THERMAL PROCESSING OF CANNED FOODS IN TIN CONTAINERS

II. NOMOGRAMS FOR GRAPHIC CALCULATION OF THERMAL PROCESSES FOR NON-ACID CANNED FOODS EXHIBITING STRAIGHT-LINE SEMI-LOGARITHMIC HEATING CURVES

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Canning procedures for perishable foods incorporate two fundamental practices: hermetic sealing of the container and commercial sterilization of the food by heat. For successful preservation the thermal process accorded a food must provide for its subjection to a proper temperature, or temperatures, for a period of time sufficiently long to produce a condition of commercial sterility. The hermetic character of the seal imposed upon the container will prevent infection of the food by spoilage organisms to which the container may subsequently be exposed. Liquid food products of an acid nature, such as the common fruit juices, may be thermally processed before the container is hermetically sealed. Most products, however, are processed after the can has been permanently closed.

Although the necessity for adequate thermal processes had long been recognized, it was not until some 20 years ago that the problem of process determination was approached on a scientific basis. Prior to that time, determination of adequate processes for any specific product had been largely a matter of reasoning by analogy from commercial experience with other products, or of "cut and try" experimentation. As the science of bacteriology became more widely applied to spoilage problems of the canning industry, it became apparent that bacteriological as well as certain physical data should be given consideration in the determination of adequate thermal processes for canned foods.

The problem of applying bacteriological and physical data to the determination of thermal process times for canned foods was first solved by Bigelow, *et al.* (1920). In the canning industry the method devised by Bigelow and his collaborators is usually referred to as the "general method." It is advantageous in that it may be applied even when the thermal death times at various temperatures and the heat-penetration data are not given in mathematical form. However,

the general method is open to the serious objection that it can be applied only when the required processing conditions, such as retort temperature, initial temperature, and can size, are identical with those of the given heat-penetration data. Furthermore, the calculations are time-consuming and laborious.

Realizing the need for a more simple and flexible method for thermal-process calculation, Ball (1923) developed a formula method for process determination. These formulas can be applied to any case wherein the major portion of the heating curve on semi-logarithmic paper approximates a straight line or two straight lines, and wherein the thermal death-time curve on semi-logarithmic paper is, or can be assumed to be, a straight line. Ball's work not only greatly extended the scope of process calculations but simplified them as well. Less time was consumed in calculating processes, and provision was made for applying the given heat-penetration data to all can sizes and retort temperatures.

It has been found that the simpler processing problems solved by the Ball method are of such importance that further simplifications appeared desirable in order to decrease the labor of calculating and to make the method available to persons without special mathematical training. The nomograms given here were constructed with this purpose in mind and essentially they provide a graphical means of carrying out the computations required in the Ball formula method. Since the use of these nomograms will require some understanding of certain terms proposed and defined by Ball, a brief explanation of them might well be included at this point.

SIGNIFICANCE OF TERMS

Slope "z" of the Thermal Death-Time Curve: By bacteriological investigation the times required to destroy certain bacteria, under specified conditions, may be obtained at various temperatures. If, for a particular organism, the thermal death times are plotted on a logarithmic scale and the corresponding temperatures on a linear scale, the points so obtained can usually be represented by a straight line. This line can be specified mathematically by the coördinates of one point and the slope of the line. The thermal death time at 121.1°C.(250°F.) is designated by F and is arbitrarily taken as the reference point.

In analytical geometry the slope of a line is usually expressed as the tangent of the angle between the line and the X-axis. On semi-logarithmic paper, however, the slope of a line is more conveniently expressed by the number of degrees Fahrenheit on the temperature scale required to traverse one logarithmic cycle on the time scale. This number will be designated by z.

In general, the value of z may be considered constant for a given strain of bacterial spores in a given medium, or conversely, the value of z will be different for different strains of spores in the same medium or for the same strain in different media. At present, very little is known concerning the factors which cause variations in z value.

Early investigations by Esty and Meyer (1922) established the "ideal" thermal death-time curve slope as 18° for spores of *Clostridium botulinum* suspended in neutral phosphate solution. Since then, further determinations have tended to confirm this value of z for spores of *Cl. botulinum* and some related putrefactive anaerobes when suspended in neutral phosphate. In food juices, however, it would appear that the slope of the thermal death-time curve for those organisms may vary over a range of about 5° or 6° on either side of the "ideal" 18° and thus cause considerable change in values obtained by process calculations.

In the absence of more reliable information the "ideal" z value, 18°, has been quite generally used for evaluation and calculation of processes. The only exceptions have been in the cases of particular foods where determinations with a certain organism had shown some other value of z to be more applicable. For this reason the nomograms here presented (Charts 1 and 2) are constructed assuming that $z = 18°$. If further investigation of thermal death-time curves so indicates, nomograms corresponding to those shown may be prepared for other values of z. The nomogram shown (Chart 3), however, provides for variation in the value of z when the problem falls in the classification IA or IIA. The instructions for the use of Chart 3 are on the chart itself. For the present, at least, if problems in the IB or IIB classifications require the use of z values other than 18°, the formula method of calculation must be resorted to until further nomograms are constructed. The classification of processing problems is discussed later in this paper.

As an example of the meaning of F and z, two hypothetical thermal death-time curves are given (Fig. 1). Curve A crosses the 250°F. line at approximately 4.3, therefore the F value is 4.3. The thermal death time at 240°F. is 20 minutes; at 255°F. the thermal death time is two minutes. In going from two to 20 minutes, however, the curve has traversed one logarithmic cycle. The z value is then $255°F. - 240°F. = 15°F.$ For curve B, it can be seen that $F = 10$, $z = 5°F.$

Heat-Penetration Factors "j" and "f_h": The rate at which heat penetrates into the food is also an important consideration in process calculations. Heat-penetration data are customarily obtained by inserting a thermocouple into the can of food in a specified position and

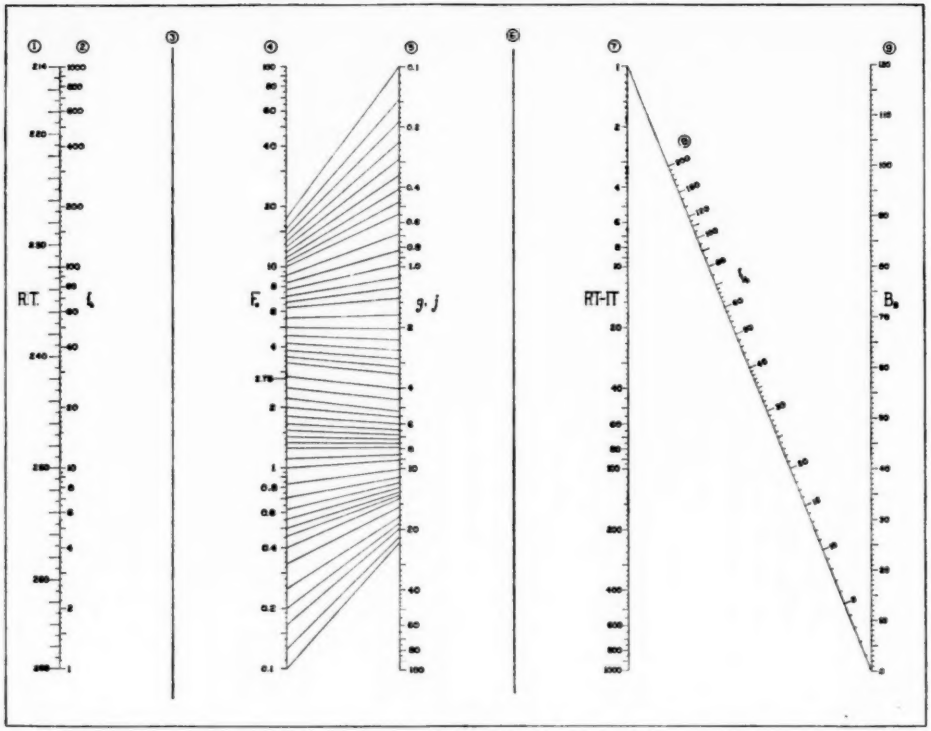

CHART 1

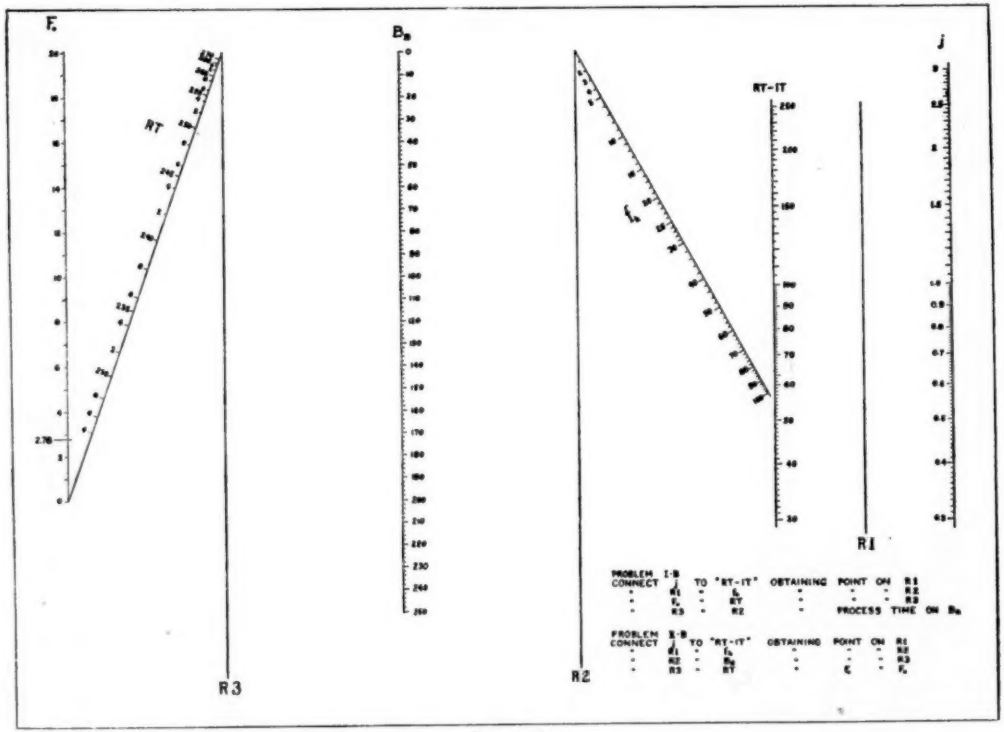
F
RT
R3
B_m
R2
RT-IT
R1
j
PROBLEM CONNECT I-B
TO "RT-IT" OBTAINING POINT ON R1
PROCESS TIME ON B_m
PROBLEM CONNECT X-B
TO "RT-IT" OBTAINING POINT ON R1

CHART 2

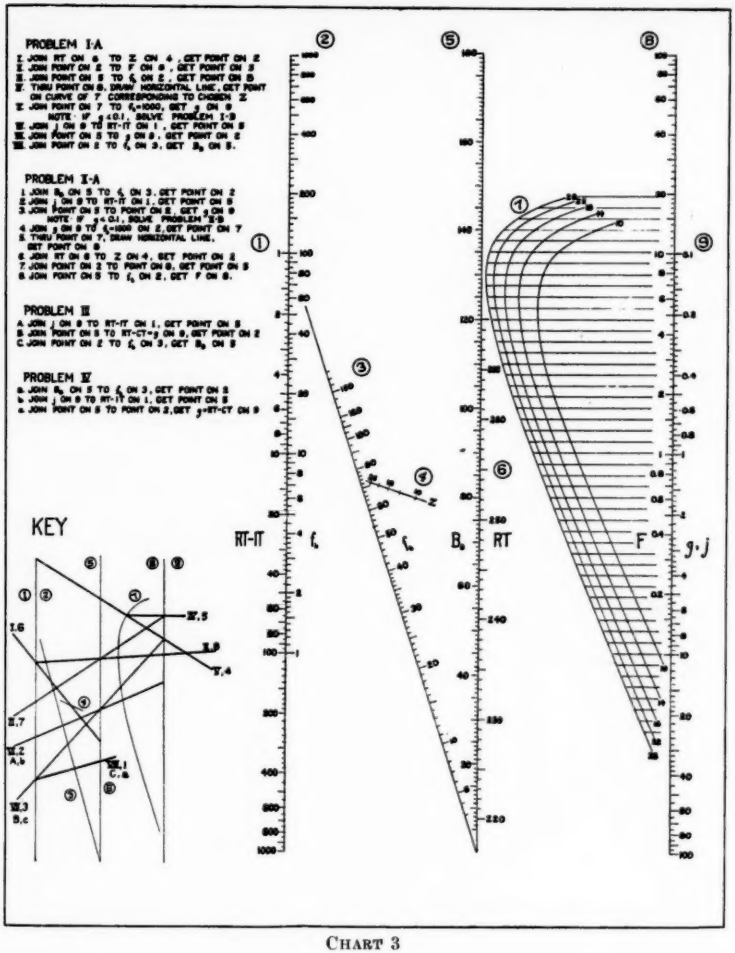

CHART 3

following the temperature changes during thermal processing in a retort.

The heat-penetration data may likewise be plotted on semi-logarithmic paper, with the temperature on the logarithmic scale and the time on the linear scale. It is necessary, however, to plot the difference between the retort temperature and the can temperature on the logarithmic scale rather than the can temperature itself. In practice an equivalent, but simpler, procedure is followed. The semi-log paper is turned through 180° so that the log scale increases from

top to bottom, and the top line on the log scale is given a value one degree below retort temperature. This is illustrated for the case of a hypothetical heating curve (Fig. 2) in which a retort temperature of 240°F. is assumed.

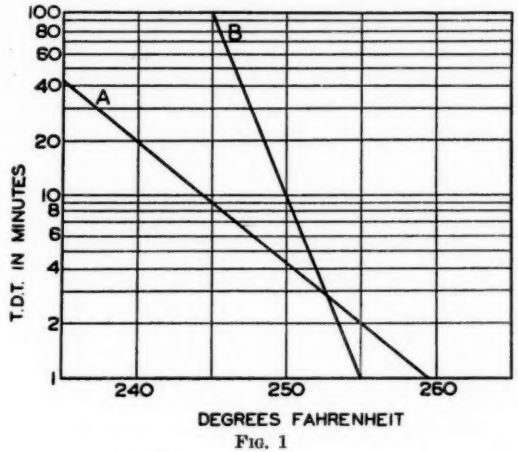

FIG. 1

For the semi-log heating curve two numbers are again necessary to specify the position of the line; f_h is the number of minutes required for the straight-line portion of the graph to traverse one log

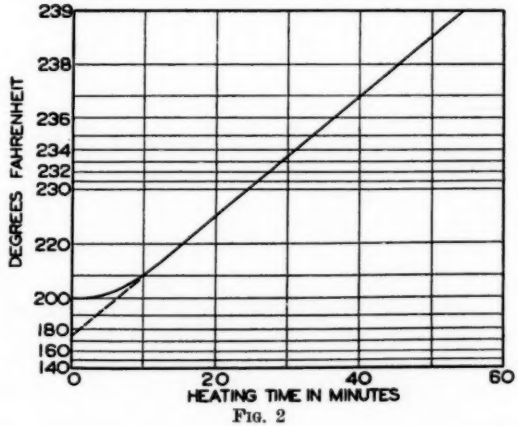

FIG. 2

cycle of temperature. A detailed discussion of the significance of f_h was given by Ball (1927). The number j is obtained by dividing the difference between retort temperature and the theoretical or

TABLE 1

f_h Conversion Factors for Conduction Heating

Given can size	Desired can size											
	202 x 214	211 x 400	300 x 407	303 x 406	307 x 302	307 x 306	307 x 400	307 x 409	307 x 510	401 x 411	404 x 414	502 x 510	603 x 700
202 x 214	1.000	1.700	2.127	2.356	2.154	2.298	2.554	2.725	2.927	3.595	3.944	5.687	8.502
211 x 400	0.588	1.000	1.251	1.386	1.267	1.352	1.502	1.603	1.721	2.114	2.320	3.344	5.000
300 x 407	0.470	0.799	1.000	1.108	1.013	1.080	1.201	1.281	1.376	1.690	1.854	2.673	3.996
303 x 406	0.424	0.722	0.903	1.000	0.914	0.975	1.084	1.157	1.242	1.526	1.674	2.414	3.608
307 x 302	0.464	0.789	0.987	1.094	1.000	1.067	1.119	1.265	1.359	1.668	1.831	2.640	3.946
307 x 306	0.435	0.740	0.926	1.025	0.938	1.000	1.111	1.186	1.274	1.564	1.716	2.475	3.699
307 x 400	0.392	0.666	0.833	0.923	0.844	0.900	1.000	1.067	1.146	1.407	1.544	2.226	3.328
307 x 409	0.367	0.624	0.781	0.865	0.791	0.843	0.937	1.000	1.074	1.319	1.448	2.087	3.120
307 x 510	0.342	0.581	0.727	0.805	0.736	0.785	0.873	0.931	1.000	1.228	1.347	1.943	2.905
401 x 411	0.278	0.473	0.592	0.656	0.599	0.639	0.711	0.758	0.814	1.000	1.097	1.582	2.365
404 x 414	0.254	0.431	0.539	0.597	0.546	0.583	0.648	0.691	0.742	0.911	1.000	1.442	2.156
502 x 510	0.176	0.299	0.374	0.414	0.379	0.404	0.449	0.479	0.515	0.632	0.694	1.000	1.495
603 x 700	0.118	0.200	0.250	0.277	0.253	0.270	0.300	0.321	0.344	0.423	0.464	0.669	1.000

pseudo-initial temperature by the difference between retort temperature and the actual or real initial temperature. The use of this ratio is made necessary by the fact that a uniform rate of heating is seldom if ever established instantaneously in a can of food during a thermal process. Either a lag or a "jump," depending upon the temperature gradients in the can, is usually recorded during the early portion of the cook. By virtue of the definition of the pseudo-initial temperature, the ratio j also compensates the changing temperature in the retort during the come-up period. The pseudo-initial temperature is a point on the extension of the straight-line portion of the heating curve having a time coördinate which is determined by the form of the retort come-up curve.

The solid line represents the actual heating curve (Fig. 2); the dotted line is an extension of the straight part of the heating curve; and f_h is 30. The pseudo-initial temperature is the temperature given by the dotted part of the curve at the beginning of the process (assuming instantaneous retort come-up). In this case it is 175°F. The value of j is found by subtracting the pseudo-initial temperature from the retort temperature and dividing this value by the difference between the retort temperature and the actual initial temperature. The j value of the heating curve (Fig. 2) is then

$$\frac{240—175}{240—200} = \frac{65}{40} = 1.62.$$

The straight-line representation of the same heating curve is then completely specified by $j = 1.62$ and $f_h = 30$.

Not all heating curves, however, are of the simple form shown. Some products have a heating curve exhibiting a sharp break followed by a permanent change in the slope. Ball (1928) has given methods for determining processes for products having this type of heating curve. Inasmuch as the nomograms given here are for use only for products having a simple heating curve, the characteristics of broken heating curves need not be discussed further.

If it is desired to calculate a process for a can size other than the one used in obtaining the heat-penetration data, it is necessary to find the value of f_h corresponding to the new can size. In the case of conduction heating, the given value of f_h can be multiplied by the conversion factor (Table 1) corresponding to the given and new can sizes. The value of f_h so obtained will be the f_h for the given product in the new can size. If the product heats mainly by convection, as is the case with products such as broths or brine-packed peas, the conversion factors (Table 2) should be used. These factors were ob-

TABLE 2

f_h Conversion Factors for Convection Heating

Given can size	Desired can size												
	202 x 214	211 x 400	300 x 407	303 x 406	307 x 302	307 x 306	307 x 400	307 x 409	307 x 510	401 x 411	404 x 414	502 x 510	603 x 700
202 x 214	1.000	1.301	1.453	1.511	1.409	1.454	1.550	1.616	1.714	1.839	1.943	2.380	2.840
211 x 400	0.768	1.000	1.116	1.161	1.083	1.117	1.190	1.242	1.317	1.413	1.493	1.829	2.182
300 x 407	0.688	0.896	1.000	1.040	0.970	1.001	1.066	1.113	1.180	1.266	1.338	1.638	1.935
303 x 406	0.662	0.861	0.961	1.000	0.932	0.962	1.025	1.069	1.134	1.217	1.286	1.575	1.879
307 x 302	0.710	0.924	1.031	1.073	1.000	1.032	1.099	1.147	1.216	1.306	1.379	1.689	2.016
307 x 306	0.688	0.895	0.999	1.039	0.969	1.000	1.065	1.111	1.179	1.265	1.336	1.637	1.953
307 x 400	0.646	0.840	0.938	0.976	0.910	0.939	1.000	1.044	1.107	1.188	1.254	1.537	1.834
307 x 409	0.619	0.805	0.903	0.935	0.872	0.900	0.958	1.000	1.060	1.138	1.203	1.473	1.757
307 x 510	0.584	0.759	0.848	0.882	0.822	0.849	0.904	0.943	1.000	1.073	1.134	1.389	1.657
401 x 411	0.544	0.708	0.790	0.822	0.766	0.791	0.842	0.879	0.932	1.000	1.056	1.294	1.544
404 x 414	0.515	0.670	0.748	0.778	0.725	0.748	0.797	0.832	0.882	0.947	1.000	1.225	1.462
502 x 510	0.420	0.547	0.610	0.635	0.592	0.611	0.651	0.679	0.720	0.773	0.816	1.000	1.193
603 x 700	0.352	0.458	0.512	0.532	0.496	0.512	0.545	0.569	0.603	0.648	0.684	0.838	1.000

tained from a table of can factors for products heating by convection given by Schultz and Olson (1938). The value of j may be considered practically invariant with respect to can size.

Sterilizing Value of a Process: One concept remains to be explained, that is, the "sterilizing value" of a process. Two processes are said to be equivalent with respect to a given organism when they are equally effective with respect to the heat destruction of that organism. The sterilizing value of a given process is defined to be the length in minutes of an equivalent process at 250°F. where the can contents immediately upon the beginning of the process attain retort temperature, are held at that temperature for the length of the process, and at the end of the process instantaneously drop to a sublethal temperature. The length in minutes of this equivalent process is often referred to as the F value. This concept is of great importance as it permits a quantitative evaluation of various processes.

Laboratory investigation, as well as commercial experience, over a period of years has provided valuable information on the F values of processes required to produce commercial sterility in many canned products. The basic criterion of commercial sterility is the effectiveness of the process with respect to spores of *Clostridium botulinum*, a toxin-producing organism. Esty and Meyer (1922) determined an "ideal" thermal death curve for spores of *Cl. botulinum* in neutral phosphate. This curve is designated by the values $F = 2.78$ minutes and $z = 18°F$. Consequently, processes having an F value of at least 2.78 minutes have been considered absolutely necessary for all non-acid canned foods unless well-checked evidence indicates that the chemical or biochemical nature of the product necessitates a revision. Actually the F values of processes required to safeguard the health of the consumer of such products as corn, peas, spinach, beans, and most meat and fish products range from 4.5 minutes upward. The presence of other more resistant spoilage organisms may require F values of six minutes or more. Selection of a proper F value for purposes of calculating a process requires a considerable amount of experience in processing studies and a thorough understanding of packing procedures as well as the nature of the product.

Classification of Processing Problems: The types of processing problems to be considered are as follows:

1. How long must a can be processed in order to destroy the spores of a particular organism?
2. What is the sterilizing value of a given process?
3. How long must a can be processed in order to reach a given temperature at the slowest heating point in the can?

4. What is the temperature at the slowest heating point in the can at the end of a given process?

The solutions to these problems may be found very readily by means of the nomograms given here. Before discussing their use, it may be well to consider the accuracy of the result obtained in using these nomograms.

There are three causes of errors in the results obtained with any nomogram. The first is the error due to careless *operation*, the second is the error due to faulty *construction* of the nomogram, and the third is the error due to the *design* of the nomogram. The first two are ordinarily unknown but the magnitude of the third can usually be determined. In the case of Chart 1, it is necessary to assume that the difference between the retort temperature and the cooling water

TABLE 3

Corrections to Be Applied to Length of Process Obtained From Chart 1

RT-CW	f_h	5	10	20	50	100	200
200	Add	0.14	0.28	0.56	1.41	2.82	5.64
190	Add	0.09	0.19	0.38	0.94	1.88	3.76
180	Add	0.05	0.09	0.19	0.47	0.94	1.88
170	.....	0	0	0	0	0	0
160	Subtract	0.02	0.03	0.07	0.18	0.36	0.71
150	Subtract	0.03	0.07	0.14	0.35	0.71	1.42
140	Subtract	0.05	0.10	0.21	0.54	1.07	2.14

temperature is 170°F. If some such assumption were not made, the resulting nomogram would be unduly complicated. Knowing the assumptions made in the design, it is possible to obtain the limits of the errors that are due to design. The corrections to be applied to the results obtained with the nomograms (Table 3) are made in such a manner as always to be on the side of safe commercial practice.

USE OF THE NOMOGRAMS

Application of the nomograms to the solution of problems of the general type listed above can best be explained by specific examples.

Problem I

What length of process (B_B) is required to destroy the spores of a given organism?

Let the retort temperature be RT, the initial temperature be IT, the resistance of the organism be F_o minutes at 250°F. Assume further that the temperature of the cooling water is 70°F. and that j and f_h are given.

Step 1: On Chart 1, find RT on scale 1, F_o on scale 4, connect RT and F_o and get a point on line 3.

Step 2: Connect the point on line 3 to f_h on scale 2 and get a point on scale 4. By following the series of lines connecting scales 4 and 5, find the point on scale 5 corresponding to the point on scale 4.

NOTE: If the point on scale 4 is *above* the top line connecting scale 4 to scale 5, it indicates that the final can temperature is less than .1°F. below retort temperature. In such a case, Chart 2 must be used. The directions are given under Problem I-B on the chart itself.

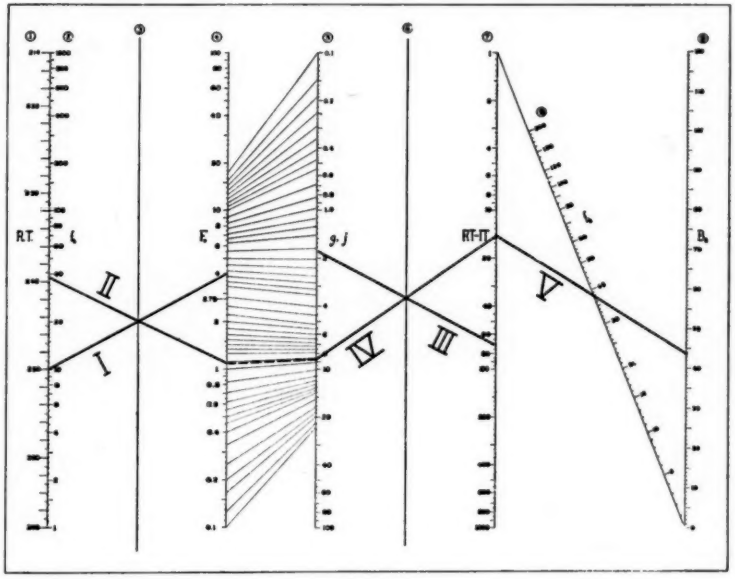

FIG. 3

Step 3: Subtract the initial temperature from the retort temperature. Find RT-IT on scale 7 and connect to j on scale 5, getting a point on line 6.

Step 4: Connect the point on line 6 to the point on scale 5 (Step 2) getting a point on scale 7.

Step 5: Connect the point on scale 7 to f_h on scale 8, find the length of process B_B on scale 9.

Example I-A, Fig. 3

What is the length of process (B_B) at 250°F. necessary to destroy spores of an organism having a resistance of four minutes at 250°F. when the slope of the thermal death-time curve is $z = 18$°F.?

Assume that the product has an initial temperature of 180°F., and that it has a "heavy" consistency, and so heats mainly by conduction.

Suppose further that the heat-penetration data ($f_h = 60$ and $j = 1.8$) have been determined for a No. 2 can (307 x 409) and that a process is desired for a No. 1 can (211 x 400). We find the f_h conversion factor for conduction heating to be .624 (Table 1). Then the value of f_h for a 211 x 400 can is $60 \times .624 = 37.4$. We are now ready to determine the length of process.

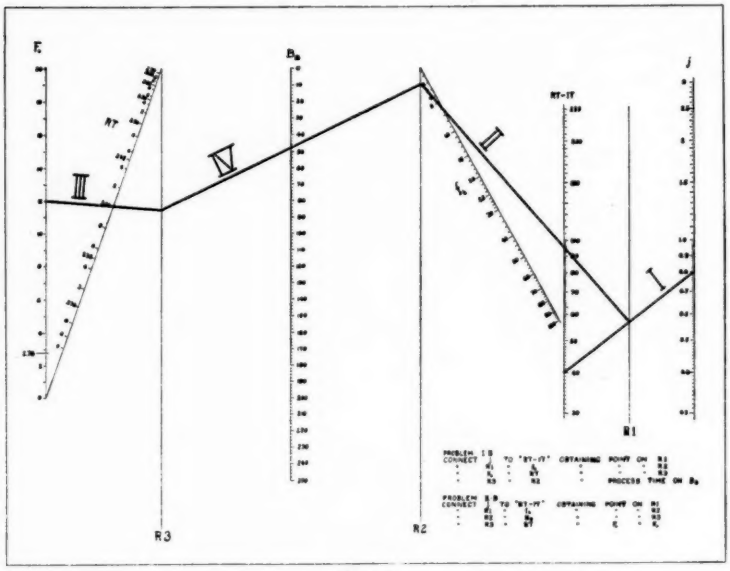

FIG. 4

Step 1: Connect RT = 250 on scale 1 to $F_o = 4$ on scale 4; get a point on line 3.

Step 2: Connect the point on line 3 to $f_h = 37.4$ on scale 2; get a point on scale 4 and the corresponding point on scale 5.

Step 3: Connect $j = 1.8$ on scale 5 to RT-IT = 250 — 180 = 70 on scale 7, get a point on line 6.

Step 4: Connect the point on line 6 to the point on scale 5 (Step 2); get a point on scale 7.

Step 5: Connect the point on scale 7 to $f_h = 37.4$ on scale 8; get the process time $B_B = 44$ on scale 9.

Example I-B, Fig. 4

What is the length of process (B_B) at 240°F. necessary to destroy spores of an organism having a resistance of 12 minutes at 250°F. when the slope of the thermal death-time curve is $z = 18$°F. and the initial temperature is 200° F.?

Assume that the product is a thin fluid, so that the heating will be mainly by convection. Suppose further that the heat-penetration data ($f_h = 5.0$ and $j = .80$) have been determined for a 211 x 400 can and that it is desired to process the product in a 300 x 407 can. The f_h conversion factor for convection heating is 1.116 (Table 2). Therefore, f_h for the 300 x 407 can is $5 \times 1.116 = 5.6$. However, if we go through Step 2, we find that the derived point on scale 4 is above the top line joining scale 4 to scale 5. Therefore, we must use Chart 2. The method of solution is shown (Fig. 4).

Problem II

What is the sterilizing value (F_o) of a given process?

Let the process be B_B minutes at a retort temperature RT; the initial temperature is IT. Assume that $z = 18$, the temperature of the cooling water is 70°F., and that j and f_h are given.

Step 1: On Chart 1, connect B_B on scale 9 to f_h on scale 8; get a point on scale 7.

Step 2: Connect j on scale 5 to RT-IT on scale 7; get a point on line 6.

Step 3: Connect the point on scale 7 (Step 1) to the point on line 6 (Step 2); get a point on scale 5 and the corresponding point on scale 4.

NOTE: If the point on scale 5 is above the top line connecting scales 4 and 5, it indicates that the can temperature is less than .1°F. below retort temperature. In such a case Chart 2 must be used. The directions are given under Problem II-B on the chart itself.

Step 4: Connect the point on scale 4 to f_h on scale 2; get a point on line 3.

Step 5: Connect the point on line 3 to RT on scale 1; get F_o on scale 4.

Example II-A, Fig. 5

What is the sterilizing value (F_o) of a 100-minute process at 242°F., if $IT = 145$, $j = 1.5$, and $f_h = 68$?

Step 1: Connect $B_B = 100$ on scale 9 to $f_h = 68$ on scale 8; get point on scale 7.

Step 2: Connect $j = 1.5$ on scale 5 to $RT\text{-}IT = 242 - 145 = 97$; get point on line 6.

Step 3: Connect the point on scale 7 (Step 1) to the point on line 6 (Step 2); get a point on scale 5 and the corresponding point on scale 4.

Step 4: Connect the point on scale 4 to $f_h = 68$ on scale 2; get a point on line 3.

Step 5: Connect the point on line 3 to RT = 242 on scale 1; get $F_o = 5.6$ on scale 4. Therefore, the process is equivalent to holding the can contents 5.6 minutes at 250°F., assuming instantaneous heating and cooling.

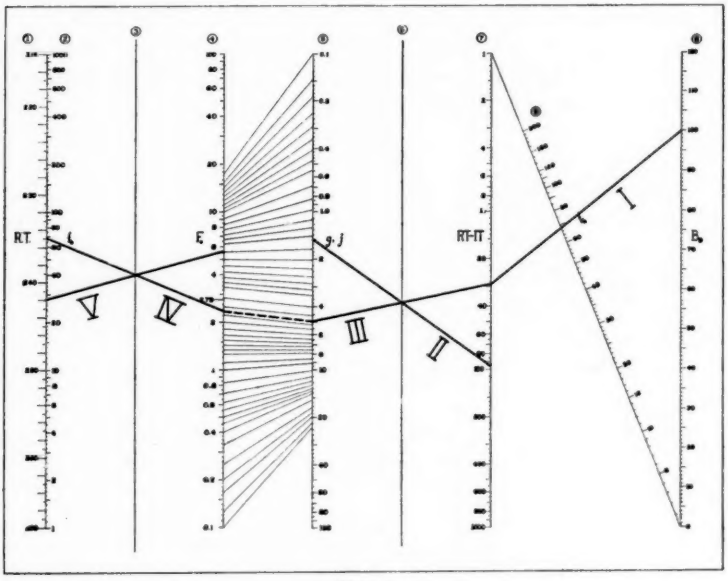

FIG. 5

Example II-B, Fig. 6

What is the sterilizing value (F_o) of a 30-minute process at 240°F. if IT = 175, j = .75, and $f_h = 10.2$?

On carrying out Steps 1, 2, and 3, it is found that this problem must be solved using Chart 2. The method of solution is shown (Fig. 6).

Problem III

What process (B_B) is required to reach a given temperature at the slowest heating point in the can? RT, IT, CT, j, and f_h are given.

Step 1: Connect RT-IT on scale 7 to j on scale 5; get point on line 6.

Step 2: Connect point on line 6 to RT-CT = g on scale 5; get point on scale 7.

Step 3: Connect point on scale 7 to f_h on scale 8; get process time on scale 9.

Example III, Fig. 7

What process (B_B) is required to reach a can temperature of 190°F. if RT = 210°F., IT = 120°F., j = 1.2, and f_h = 47?

Step 1: Connect RT-IT = 210 — 120 = 90 on scale 7 to j = 1.2 on scale 5; get point on line 6.

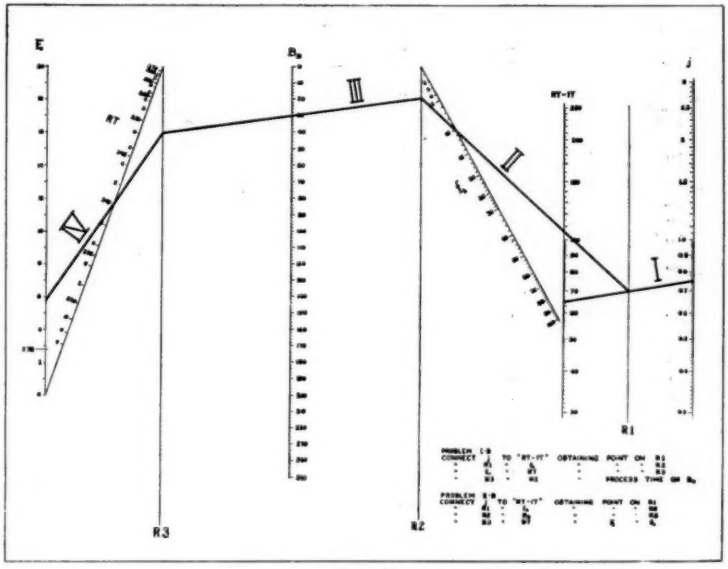

FIG. 6

Step 2: Connect point on line 6 to RT-CT = 210 — 190 = 20 on scale 5; get point on scale 7.

Step 3: Connect point on scale 7 to f_h = 47 on scale 8; get process of 34 minutes on scale 9.

Problem IV

What is the temperature (CT) at the slowest heating point in the can at the end of a given process? RT, IT, B_B, j, and f_h are given.

Step 1: Connect B_B on scale 9 to f_h on scale 8; get point on scale 7.

Step 2: Connect RT-IT on scale 7 to j on scale 5; get point on line 6.

Step 3: Connect point on line 6 to point on scale 7 (Step 1); get RT-CT on scale 5. Then CT = RT-(RT-CT).

Example IV, Fig. 8

What is the temperature (CT) at the slowest heating point in a can after an 82-minute process at 237°F.? IT = 75°F., j = 1.4, f_h = 110.

Step 1: Connect B_B = 82 on scale 9 to f_h = 110 on scale 8; get point on scale 7.

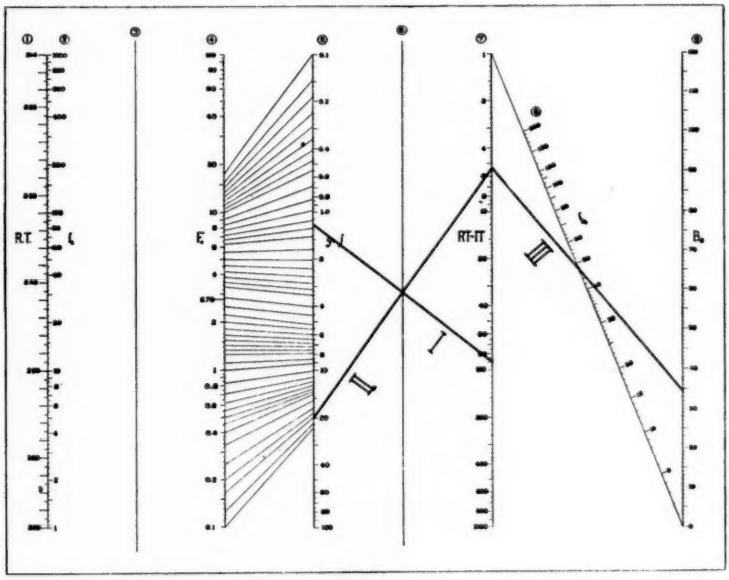

FIG. 7

Step 2: Connect RT-IT = 237 — 75 = 162 on scale 7 to j = 1.4 on scale 5; get point on line 6.

Step 3: Connect point on scale 7 (Step 1) to point on line 6 (Step 2); get RT-CT = 42 on scale 5. Then CT = RT-(RT-CT) = 237 — 42 = 195°F.

In exceptional cases it may happen that a point falls outside the range of a scale. The following rules often can be applied to these unusual cases:

For Chart 1:

1. f_h on scale 8 and B_B on scale 9 can both be multiplied by the same factor.

2. If j on scale 5 is multiplied by a given factor, RT-IT on scale 7 must be divided by the same factor.
3. f_h on scale 2 and F_o on scale 4 can both be multiplied by the same factor.

For Chart 2: If j is multiplied by a given factor, RT-IT must be divided by the same factor.

As an illustration, the following purely hypothetical problem will be solved. What is the length of process if $F_o = .08$, $j = 200$, $f_h =$

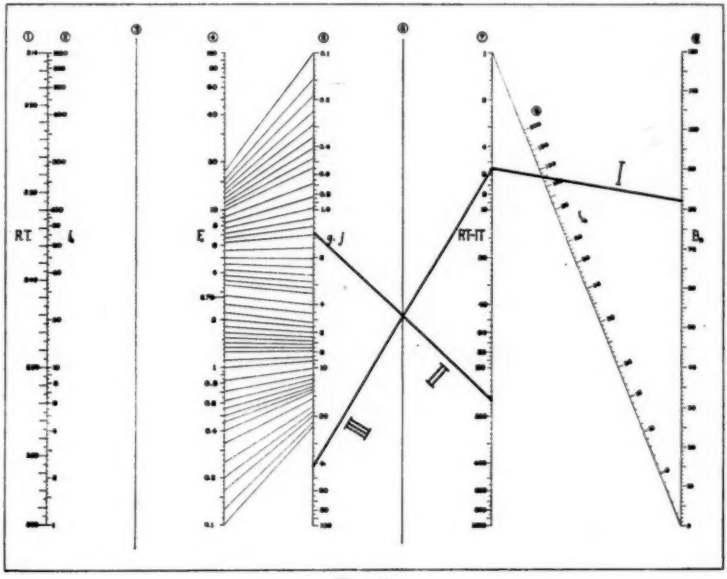

FIG. 8

60, RT = 230, and IT = 180? (This j value of 200 is taken purely for the purpose of exposition since a value of this magnitude is never encountered commercially.)

As usual, Chart 1 should be used to start the solution.

Step 1: Connect RT = 230 on scale 1 to $F_o = .08$ on scale 4. Since this point is not on the scale, multiply both F_o and f_h by 10; then use the point $F_o = .8$ to get a point on line 3.

Step 2: Connect the point on line 3 to $f_h = 60 \times 10 = 600$ on scale 2; get points on scales 4 and 5.

Step 3: Connect RT-IT = 230 — 180 = 50 on scale 7 to j = 200 on scale 5. Since j = 200 is not on scale 5, we must divide j by 10

and multiply RT-IT by 10. Therefore, connect $j = 20$ to RT-IT = 500 and get a point on line 6.

Step 4: Connect this point on line 6 to the point on scale 5 (Step 2); get point on scale 7.

Step 5: Connect the point on scale 7 to $f_h = 60$ on scale 8. If this is done, the desired point will not fall on scale 9. Therefore, take $f_h = 30$ and get $B_B = 82$ on scale 9. Since f_h is half the given value, the obtained value of B_B must be doubled, giving a process of 164 minutes at 230°F.

SUMMARY

Nomograms have been constructed for the graphic calculation of thermal processes for non-acid foods exhibiting straight-line, semi-logarithmic heating curves. Complete descriptions of the use of the nomograms are given together with illustrative examples.

ACKNOWLEDGMENT

The authors wish to acknowledge the helpful criticisms and suggestions furnished by Mr. R. H. Lueck and Drs. C. O. Ball and R. W. Pilcher, of the Research Department of the American Can Company.

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DETERMINATION OF THE HEAT RESISTANCE OF NON-SPORE-FORMING BACTERIA

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(Received for publication, May 6, 1938)

Although considerable literature has been accumulated on the heat resistance of non-spore-forming bacteria, our knowledge in this field is still inadequate, particularly in comparison with that of the heat resistance of the spore-forming bacteria. No attempt will be made here to completely assemble the literature,[2] as reviews of certain phases of the general problem have been published by Rosenau (1908), North and Park (1927), Robertson (1927), Esty (1928), Reichel (1931), Hampil (1932), and Rahn (1932). Methods which have been used in the determination of heat resistance include the inoculation and heating of a series of cotton-plugged tubes containing some fluid, sometimes requiring enrichment prior to incubation; sealed glass ampules containing the suspension to be tested; and the determination of death rates.

It is believed that many of the factors involved in the heat resistance of the spore-forming bacteria also influence that of non-spore-forming bacteria. In addition, there is the problem of supplying a new suspension of test cells for each series of experiments, since standardized suspensions cannot be prepared and used over a long period of time. The lower heat resistance exhibited by these organisms possibly exaggerates the errors due to contamination of tubes. The following comparative investigation of procedures used previously was undertaken to determine the possibility of obtaining more accurate data.

EXPERIMENTAL PROCEDURE

In the present study two modifications of the open-tube method were examined: (1) four or eight tubes heated for various closely spaced periods of time at given temperatures [57.3°C.(135°F.), 60°C. (140°F.), 62.8°C.(145°F.)] and (2) 25 tubes heated at four or five widely spaced periods of time at various temperatures [54.5°C. (130°F.), 57.3°C.(135°F.), 60°C.(140°F.), and 62.8°C.(145°F.)].

[1] Now with the George Williams Hooper Foundation for Medical Research, University of California, San Francisco.

[2] A more detailed review of the literature is available in the thesis of the senior author presented in partial fulfillment of the requirements for the degree of Master of Arts at the University of California, December, 1937.

Death rates for the same organism were also determined by the method of Levine, Buchanan, and Lease (1927).

In the experiments by Clark (1928) using tubes, m/15 sodium potassium phosphate buffer solution was used as the heating fluid. Forty-eight-hour cultures of *Escherichia coli* grown on nutrient agar at 37°C.(98.6°F.) were suspended in 10 c.c. of buffer solution and filtered through sterile cotton to obtain clump-free suspensions. The number of cells was determined (hemocytometer count) and the suspension diluted to the required number of cells.

The tubes containing phosphate buffer solution were placed in the constant temperature bath and allowed to reach the temperature of the bath.[3] Following inoculation, heating, and cooling, the tubes were enriched with .5 c.c. of concentrated broth, incubated at 37°C. (98.6°F.), and observed for growth.[4]

The method used for the determination of death rates was essentially that of Levine, Buchanan, and Lease (1927). The heating fluid and suspensions of organisms were prepared in the same manner as for the tube technic. The sterilized heating flasks were placed in the bath and the contents (100 c.c. of buffer solution) allowed to reach the temperature of the bath before inoculation. Serial-dilution counts were made by plating at the initial time and at intervals during the heating. The average values of the counts were used in the calculation of rate constants and in plotting the data.

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[3] By this method the lag factor for the time required for the tube of heating fluid to reach equilibrium with the water bath is eliminated. Obviously this method could not be applied when sealed tubes are employed but it was introduced in this study in the hope that the exclusion of this lag factor would be an aid in securing consistent data.

[4] Usually the great majority of positive tubes appeared during the second and third days of incubation. The question of delayed growth, however, must be considered since Burke, Sprague, and Barnes (1925) reported that the growth of *Escherichia coli*, when only a few cells were present in the inoculum, was delayed in some cases as long as two weeks. In this work many of the tubes in the earlier experiments were observed for growth for this length of time. In no case was the appearance of additional positive tubes observed after three to four days. Consequently in the later experiments, the tubes were discarded after five to six days' incubation.

Occasionally contamination of heated tubes occurred. However, when contamination occurred, the contaminant was one very different in growth characteristics from the organisms studied in that a heavy pellicle was formed on the surface of liquid media. Thus, no difficulty was encountered in recognizing contamination. In many of the experiments all positive tubes or those in the longer-time periods were confirmed by sub-culturing on eosin-methylene-blue agar. In no case did a positive tube upon sub-culture fail to be confirmed, except those showing growth of the pellicle-forming organisms. The use of this confirmatory procedure was finally discontinued and all positive tubes showing clear liquid but having surface growth in the form of a pellicle were considered negative. In general, very few contaminated tubes were encountered, probably less than .01 per cent throughout the entire study.

TUBE EXPERIMENTS

The results obtained when four tubes were heated at each time interval were not satisfactory, because, as may be observed (Table 1), the end points at the same temperature vary considerably. Also presented in this table are data for some of the eight tube experiments (Runs 33 and 35). Although the results of these experiments are still unsatisfactory, they indicate that the thermal death time at 57.3°C.(135°F.) for the strain of *Escherichia coli* used lies between 20 and 30 minutes. Results obtained for higher temperatures exhibited similar irregularities. The "skips" observed in these experiments confirm the observations of Lembke (1937) with *Escherichia coli*.

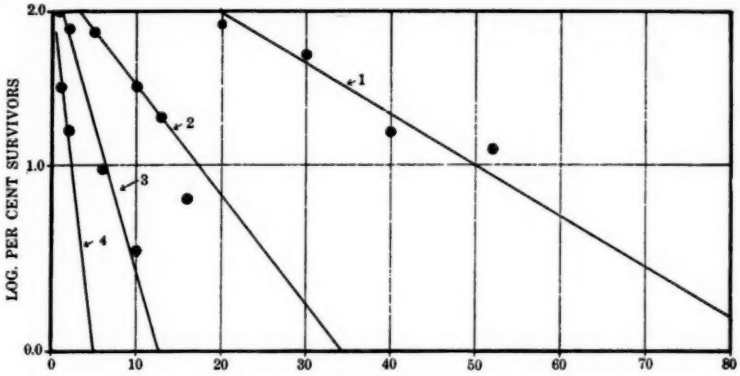

FIG. 1. *Escherichia coli*. Logarithms of percentage of tubes containing surviving cells after heating in phosphate buffer at pH 7.
[1] 54.5°C.(130°F.). [2] 57.3°C.(135°F.). [3] 60.0°C.(140°F.). [4] 62.8°C.(145°F.)

Several experiments were performed using the method of Esty and Williams (1924) in which 25 tubes were heated at each of four heating periods and four temperatures. When the logarithm of the percentage of tubes showing growth is plotted against time, a straight line is obtained (Fig. 1). The lines so established were extended to the point of apparent one-per cent survival and the time values obtained used to determine the slope of the thermal death-time curve according to the method of Bigelow (1921). The slope of the curve (Fig. 2) can be determined with fair accuracy using time values determined by extrapolation from the curves of Fig. 1.

DEATH-RATE EXPERIMENTS

On the assumption that destruction by heat occurs at a rate proportional only to the numbers of organisms present, according to

TABLE 1

Escherichia Coli Heated in Tubes of Phosphate Buffer, pH 7, at 57.3°C.(135°F.)[1]

Run No.	Growth	Time in minutes																						
		7	8	9	10	11	12	14	16	17	18	20	22	23	25	26	27	30	33	35	36	40	45	50
18	Positive[2]	1	2	0	1	3	1	1	0	...	0	...	...	...	...	...	...	...	...	...	...	...	...	...
	Negative[2]	3	2	4	3	1	3	3	4	...	4	...	...	...	...	...	...	...	...	...	...	...	...	...
21	Positive	...	2	1	0	1	0	0	0	...	0	0	1	...	...	...	...	...	...	...	...	...	...	...
	Negative	...	2	3	4	3	4	4	4	...	4	4	3	...	...	...	...	...	...	...	...	...	...	...
27	Positive	...	2	1	2	3	0	1	0	...	0	0	0	...	0	...	0	0	0	...	0	0	0	0
	Negative	...	2	3	2	1	4	3	4	...	4	4	4	...	4	...	4	4	4	...	4	4	4	4
30	Positive	...	1	2	0	3	1	1	2	...	0	0	0	...	0	...	0	0	0	...	0	0	0	0
	Negative	...	3	2	4	1	3	3	2	...	4	4	4	...	4	...	4	4	4	...	4	4	4	4
33	Positive	...	3	2	1	2	1	0	1	...	1	0	3	...	3	...	1	0	0	...	0	0	0	0
	Negative	...	5	6	7	6	7	8	7	...	7	8	5	...	5	...	7	8	8	...	8	8	8	8
35	Positive	...	1	1	0	...	1	0	...	1	...	1	...	1	...	0	...	0	...	0	...	0	...	...
	Negative	...	7	7	8	...	7	8	...	7	...	7	...	7	...	8	...	8	...	8	...	8	...	...

[1] Number of cells—1,000,000 per c.c. [2] Positive and negative refer to the number of tubes showing the presence or absence of surviving cells.

Madsen and Nyman (1907), the rate constants calculated for the interval between any two experimental time values *(k)* and for the interval between zero time and any other given time *(k_1)* are given (Table 2). It will be observed that there is greater uniformity in the case of k_1 than k. Upon plotting the data, however, and drawing lines representing values calculated from the average values of the rate constants, in general, those calculated from the average value of k fitted the experimental points better than those from average values of k_1, consequently the values of k were used for plotting and in calculating the times of .01-per cent survival (Table 3). Although

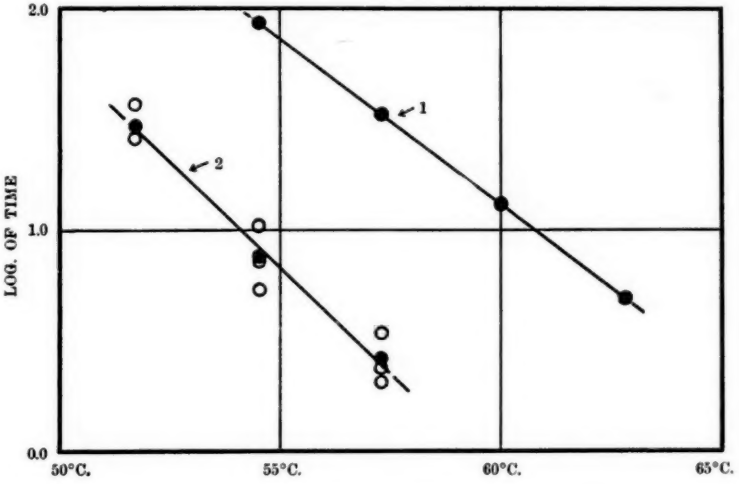

FIG. 2. Thermal death-time curves of *Escherichia coli*.
[1] Multiple-tube data (Fig. 1). [2] Death-rate data (Table 3).

natural logarithms were used for calculating the rate constants, logarithms to the base 10 were used in plotting data.

The numbers of organisms surviving at the various time intervals were used to calculate the percentage survival at a given time. The logarithms of these values were used in plotting the data. Data for *Escherichia coli* are presented (Table 2 and Fig. 3). A summary of the calculated times of .01-per cent survival and the values of the rate constants for the above-mentioned and other experiments is presented (Table 3). These and also other data indicate that different suspensions of the same organism heated at the same temperature exhibit differences in the rate of death. In some cases there seems

to be a correlation between increase in the numbers of cells in the initial inoculum and decrease in the rate of death. These observations confirm the results of Watkins and Winslow (1932). However,

TABLE 2

Death Rates of Escherichia Coli Heated in Phosphate Buffer at pH 7

Temperature	Time	Number of cells per c.c.	k	k_1	Survival	Log. survival
	min.				*pct.*	*pct.*
	0	650,000	.....	.....	100.0	2.00
	2	240,000	0.500	0.500	37.0	1.57
51.7°C.	4	172,000	0.168	0.332	26.4	1.42
(125°F.)	6	98,000	0.288	0.315	15.0	1.18
	10	9,000	0.599	0.426	1.5	0.18
	15	3,100	0.226	0.339	0.48	$\bar{1}$.68
	20	500	0.368	0.359	0.077	$\bar{2}$.89
	Average value.....		0.358	0.379		
	0	600,000	.....	.....	100.0	2.00
54.5°C.	1	170,000	1.26	1.26	28.3	1.45
(130°F.)	2	14,000	2.49	1.88	2.34	0.37
	3	3,200	1.48	1.74	0.53	$\bar{1}$.72
	Average value.....		1.74	1.63		
	0	840,000	.....	.....	100.0	2.00
57.3°C.	0.5	80,000	4.70	4.70	9.8	0.99
(135°F.)	1	2,700	6.78	5.73	0.5	$\bar{1}$.70
	2	1,100	1.08	3.32	0.2	$\bar{1}$.30
	Average value.....		4.18	4.55		

in other cases this correlation was not observed. Further work with non-spore-forming bacteria is needed because of the importance of this factor in problems of heat sterilization.

In order to determine if the slopes of thermal death-time curves can be established with data from death-rate experiments, the data

TABLE 3

Summary of Times of .01-Per Cent Survival and of Rate Constants

Organism	From Table No.	51.7°C.(125°F.)		54.5°C.(130°F.)		57.3°C.(135°F.)	
		Time of .01-per cent survival	Average k	Time of .01-per cent survival	Average k	Time of .01-per cent survival	Average k
		min.		*min.*		*min.*	
Esch-	2	25.2	.358	5.28	1.74	2.2	4.18
erichia	[1]	35.7	.258	7.13	1.29	3.44	2.68
coli	[1]	23.8	.387	10.8	0.852	2.0	4.60

[1] Values were taken from data not tabulated in this paper.

from three experiments on the death rate of *Escherichia coli* are presented (Fig. 2). All of the time values for .01-per cent survival are plotted, but the line drawn represents the average of all three experiments. It will be observed that there is less regularity in these experiments than in the data obtained with the technic of Esty and Williams (Fig. 2); however, the average times establish the

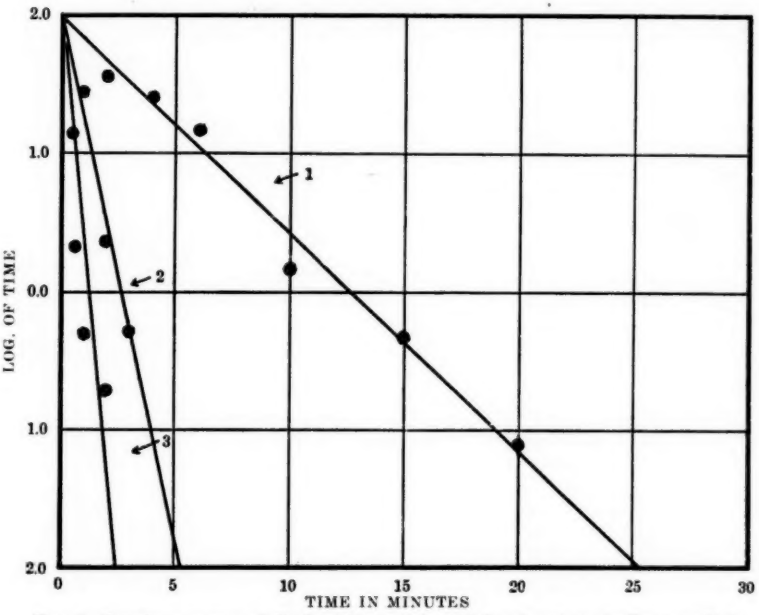

FIG. 3. Survivor curves of *Escherichia coli* heated in phosphate buffer at pH 7. [1] 51.7°C.(125°F.). [2] 54.5°C.(130°F.). [3] 57.3°C.(135°F.).

line with a fair degree of accuracy. The slope is approximately the same as obtained from the data of the other technic, although a lower heat resistance is indicated.

DISCUSSION

In this comparative study of the technics of determination of heat resistance of non-spore-forming bacteria, the results obtained by means of the tube technics indicate that close spacing of heating periods is necessary. The necessity for long heating periods and the heating of several tubes at each heating period is also apparent. Much of the earlier work on the heat resistance of non-spore-forming bacteria can be criticized because of failure to observe these essential points of technic. The results of the death-rate experiments indicate

that variations in the initial inoculum and in the velocity of death may be responsible for some of the "skips" observed.

The choice of technic to use in determining heat resistance should depend upon the use for which the data obtained are to be used. If only approximate data are required, it would be sufficient to heat a few tubes at comparatively widely spaced heating periods. In no case would it be advisable to heat a single series of tubes. If more accurate data are desired, it is suggested that usually 10 tubes and not less than five be heated at each time period. The experiment should be repeated to make certain that the maximum heat resistance has been determined. In some cases comparative data are desired, for example, data to calculate "food factors," as given by Tanner (1932, page 538), or to establish the slope of the thermal death-time curves. For these purposes more exact comparisons can be made by using the times of survival of cells in one per cent of the tubes obtained by plotting the results of the multiple-tube technic of Esty and Williams (1924). These calculations could also be made with the calculated times of .01 per cent of the cells obtained from death-rate data, which Williams and Merrill (1937) have suggested for the calculation of "food factors." In the present work, however, this method did not give as satisfactory thermal death-time curves for *Escherichia coli* as did the data of the multiple-tube technique.

ACKNOWLEDGMENT

The writers wish to acknowledge the suggestions given by Dr. M. A. Joslyn of this Division and by members of various industrial laboratories. Thanks are due the Research Department of the American Can Company for the loan of the constant temperature water bath employed in these experiments.

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VITAMIN C CONTENT OF SAUERKRAUT[1]

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(Received for publication, May 7, 1938)

Raw vegetables, including cabbage, have long been known to have certain antiscorbutic values. Cabbage is one of the vegetables that lends itself admirably to preservation by fermentation. Immunity to scurvy among certain peoples has been attributed to the use of sauerkraut in their diet.

From a review of recent papers there seems to be some question as to the vitamin C content of kraut. Ellis, Steenbock, and Hart (1921) noted that sauerkraut is relatively free of vitamin C and stated that the fermentation process resulted in the destruction of the vitamin. Wedgwood and Ford (1924) found kraut juice ineffective in preventing scurvy. Clow, Marlatt, Peterson, and Marten (1929) found about one-half as much vitamin C in sauerkraut as in raw cabbage. Lawrow and Jarussowa (1930) noted a low vitamin C content in bulk kraut. Parsons (1930) found that protection of sauerkraut from air caused a retention of the vitamin. Clow, Parsons, and Stevenson (1930) noted considerable variation among six brands of commercial kraut. Parsons and Horn (1933) could find no correlation between the variation in vitamin C content of four brands of canned kraut and the methods of manufacture.

The studies reported here were conducted to note the correlation between vitamin C content of kraut and the past history of the product. This was made possible by the development of rapid titration methods for vitamin C (ascorbic acid).

EXPERIMENTAL PROCEDURE

The analytical method employed was that of Bessey and King (1933) as modified by Mack and Tressler (1937). Samples of sauerkraut were drained on a coarse screen for one minute. Ten ml. of the juice was mixed with 40 to 50 ml. of eight-per cent acetic acid and diluted with water to a volume of 100 ml., from which aliquots were taken for titration or subsequent treatment. Twenty-gram samples of the drained solids were macerated with 40 ml. of eight-per

[1] The authors wish to express their appreciation to H. B. Hoeffler for the care taken in performing some of the analytical work presented in this paper. Approved by the Director of the New York State Agricultural Experiment Station for publication as Journal Paper No. 264, April 23, 1938.

cent trichloracetic acid. The liquid was separated by centrifuging; the solid residue was rinsed successively with 30, 15, and 10 ml. of eight-per cent acetic acid; and the combined centrifugates were made to 100 ml. The freshly cut cabbage was extracted in the same way with five-per cent sulfuric acid.

Each of the above acids was always made two per cent with respect to metaphosphoric acid to inhibit catalytic oxidation by copper. Tests indicated that these procedures insured a complete extraction of ascorbic acid and prevented any oxidation during the extraction. In all cases in which a slight oxidation might have occurred during the fermentation process, the dehydro-ascorbic acid was determined by reducing it with hydrogen sulfide previous to titration.

TABLE 1

Vitamin C Content of Freshly Cut Cabbage

Date cut	Ascorbic acid
	mg. per gm.
August 9, 1935	.38
August 14, 1935	.38
August 15, 1935	.36
August 19, 1935	.44
September 5, 1935	.34
September 12, 1935	.41
September 15, 1935	.41
October 22, 1935	.31
October 22, 1935	.33

In such an investigation it is obvious that the error in sampling may be far greater than any error in the analytical method. It is not possible to obtain samples of all the cabbage that goes into a vat nor can samples of the solids be obtained after the vat is closed. A sharpened iron pipe with holes on the side near the end was inserted into vats of kraut to secure samples of juice. After the juice had run into the pipe, the sample was withdrawn by means of a rubber tube inserted into the pipe. Samples of canned kraut or raw kraut naturally represent only a small portion of a whole vat.

Freshly Cut Cabbage: Nine samples of freshly cut cabbage obtained from various kraut factories were examined at intervals throughout the 1935 season. These have shown a variation in ascorbic acid content from .31 to .44 mg. per gram of cabbage (Table 1). Gould, Tressler, and King (1936) showed a wider variation than this but studied different varieties comparing early with late cabbage.

Raw Kraut: Seventy samples of raw kraut obtained from various kraut factories and at various times throughout the year were examined. A number of these were tested for total acid and salt. The

TABLE 2

Vitamin C Content of Raw Kraut

Age of kraut	Acidity as lactic acid	Ascorbic acid content in		
		Juice		Solids
		Before reduction	After reduction	Before reduction
mo.	*pct.*	*mg. per gm.*	*mg. per gm.*	*mg. per gm.*
¼	.....	.08	.13	.....
¼	.....	.35	.....	.22
½	.....	.07	.14	.....
½	1.82	.39	.45	.....
½	1.53	.38	.46	.....
½	1.53	.28	.29	.....
½	1.35	.31	.33	.....
½	1.04	.09	.09	.....
½	0.73	.15	.16	.....
1	.....	.35	.....	.21
1	2.07	.41	.....	.36
1	1.78	.45	.....	.39
1	1.61	.46	.....	.39
1	.....	.41	.....	.33
1	1.40	.35	.....	.....
1	1.62	.35	.....	.....
1	1.82	.38	.....	.....
1	1.83	.43	.....	.37
1	1.62	.33	.....	.31
1	1.62	.33	.....	.29
1	1.89	.44	.....	.37
1	.....	.35	.....	.21
1	.....	.31	.....	.21
1	.....	.25	.26	.....
1	.....	.11	.12	.....
1	.....	.17	.18	.....
1	.....	.13	.15	.....
1	.....	.23	.26	.....
1	.....	.35	.38	.....
1	.....	.39	.39	.....
1	.....	.05	.09	.....
1	1.24	.35	.40	.....
1	1.60	.29	.33	.....
1	1.30	.38	.46	.....
1	1.29	.35	.44	.....
1	1.13	.37	.40	.....
1	.....	.....	.....	.20
1	1.69	.38	.40	.....
1	1.32	.40	.51	.....
1	1.02	.40	.42	.....
1	1.20	.38	.39	.....
1	0.98	.39	.43	.....
1	1.27	.05	.08	.20
1	.....	.09	.11	.....

TABLE 2 *(Concluded)*

Vitamin C Content of Raw Kraut

Age of kraut	Acidity as lactic acid	Ascorbic acid content in		
		Juice		Solids
		Before reduction	After reduction	Before reduction
mo.	*pct.*	*mg. per gm.*	*mg. per gm.*	*mg. per gm.*
1	1.69	.33	.41	.....
1	1.31	.32	.42	.....
1	1.68	.33	.39	.....
1	1.71	.32	.45	.....
1	1.96	.39	.44	.....
1	1.90	.34	.40	.....
1	1.82	.40	.43	.....
1	1.52	.39	.41	.27
1	1.69	.31	.33	.25
Average, 1 month or less		.310	.322	.290
1½	1.01	.08	.09	.....
1½	1.14	.01	.01	.....
2	.....	.20	.....	.11
2	1.85	.37	.....	.35
2	1.00	.40	.....	.....
3	2.10	.33	.....	.31
Average, 1-3 months		.232		.257
7	.....	.29 [1]	.....	.....
7	.....	.04 [1,2]	.....	.....
8	.....	.02 [1,2]	.....	.....
8	.....	.02 [2,3]	.....	.....
9	.....	.05 [3]	.....	.....
9	.....	.02 [2,3]	.....	.....
9	.....	.02 [2,3]	.....	.....
10	2.50	.20	.....	.17
10	2.13	.11	.12	.....
10	2.15	.23	.23	.....
11	2.05	.13	.....	.11
Average 7-11 months		.103		.140

[1] Juice drained from tank. [2] Samples of juice from edge of vats of kraut. [3] Juice not drained from tank.

ascorbic acid content varied in these samples from .01 to .51 mg. per gram of juice and .11 to .39 mg. per gram of solids (Table 2). No correlation could be noted between total acidity or salt, and the ascorbic acid content. On the other hand, a rough correlation could be noted between the amount of vitamin C and the age of the kraut. The average of determinations on all samples of kraut less than one month old was .310 mg. per gram, while those seven months or older averaged only .103 mg. per gram. Variation from these averages is great; the lowest amount of vitamin C, .01 mg. per gram of juice, was noted in a sample one and one-half months old. Another sample,

10 months old, contained .23 mg. per gram of juice. Therefore, the statement that old kraut contains less vitamin C than new kraut should be considered a general statement with many variations.

Canned Kraut: A large proportion of the kraut used at present is marketed in cans. Thirty-six samples of commercial kraut from various manufacturers canned in No. 2 plain tin cans were examined. In a majority of cases the past history of the kraut was known.

TABLE 3

Vitamin C Content of Canned Kraut

Approximate age of kraut when canned	Approximate time after canning	Acidity as lactic acid	Salt	Ascorbic acid content	
				Juice	Solids
mo.	*mo.*	*pct.*	*pct.*	*mg. per gm.*	*mg. per gm.*
1	1	1.42	.....	.28	.26
1	2	1.31	1.65	.25	.23
1	6	.....	.....	.24	.23
1½	0	1.04	.....	.25	.22
1½	1	1.03	1.55	.24	.22
1½	4	.....	.....	.24	.21
1½	10	.....	.....	.25	.15
1½	10	.....	.....	.26	.19
2	1/10	1.05	1.49	.13	.13
2	10	1.55	1.35	.26	.31
2	8	0.94	2.22	.19	.17
2	8	0.96	2.40	.18	.17
2	8	1.09	2.05	.19	.20
2	8	1.10	1.98	.20	.20
3	7	1.20	2.16	.17	.....
3	7	1.10	1.96	.12	.13
4	6	1.29	1.90	.21	.21
4	6	1.30	1.93	.21	.19
5	5	.....	.....	.18	.13
5	5	.....	.....	.15	.12
5	5	1.55	1.84	.11	.10
5	5	1.67	1.78	.10	.09
6	9	1.39	1.11	.20	.21
6	4	0.89	1.67	.10	.10
6	4	0.98	.....	.10	.11
7	3	1.29	2.05	.18	.18
8	0	1.10	1.81	.16	.18
9	1/10	1.71	1.64	.12	.11
10	1/6	1.05	1.58	.14	.13
10	1/6	1.11	1.55	.15	.12
10	1/3	1.18	1.61	.21	.21
....	....	1.20	1.55	.17	.15
....	....	1.24	1.55	.17	.17
....	0	1.40	.....	.27	.23
....	....	.....	.....	.15	.14
....	....	.....	.....	.15	.14

Variations in ascorbic acid content from .09 to .31 mg. per gram of juice or solids have been noted (Table 3). In the majority of samples the content in the solids was slightly below that in the juice. Again no correlation could be noted with the total acidity or the total salt. As in the case of raw kraut, it may be noted that the

TABLE 4

Loss of Vitamin C (Ascorbic Acid) Content of Kraut During Canning

Source of sample	Date of canning	Ascorbic acid content		Acidity as lactic acid	Salt
		Solids	Juice		
		mg. per gm.	*mg. per gm.*	*pct.*	*pct.*
Raw kraut	Dec. 23, 1935	.40	.40	.....	.....
Mixing vat		.20	.....	.....	.....
Filling table		.15	.14	.....	.....
Preheater		.....	.....	.....	.....
Preheater water added		.12	.13	1.00	.....
Cans					
Raw kraut	Jan. 11, 1936	.35	.37	.....	.....
Mixing vat		.....	.11	.....	.....
Filling table		.19	.15	1.64	1.78
Preheater		.10	.09	1.43	1.68
Preheater water added		.11	.12	1.37	1.57
Cans		.11	.12	1.64	1.70
Raw kraut	Jan. 9, 1936	.20	.11	.....	.....
Mixing vat		.....	.14	.....	.....
Filling table		.14	.15	.....	.....
Preheater		.11	.14	.....	.....
Preheater water added		.11	.12	.....	.....
Cans		.13	.13	.....	.....
Raw kraut	Jan. 9, 1936	.....	.04	1.38	2.31
Mixing vat		.....	.....	.....	.....
Filling table		.....	.....	.....	.....
Preheater		.....	.03	1.28	2.31
Preheater water added		.....	.....	.....	.....
Cans		.....	.03	1.20	2.31
Raw kraut	Feb. 11, 1937	.24	.28	1.95	.....
Mixing vat		.....	.....	.....	.....
Filling table		.18	.18	.....	.....
Preheater		.....	.....	.....	.....
Preheater water added		.....	.....	.....	.....
Cans		.16	.16	.....	.....

ascorbic acid content bears a relationship to the age of the kraut when canned. Of 31 samples of which the past history was known, eight samples represent kraut canned less than two months from the time of packing, six samples canned two months, and 17 canned more than two months. The averages of these were .232, .194, and .149 mg. of ascorbic acid per gram of juice or solids.

From these results one may note an apparent correlation between vitamin C content and age of the kraut when canned. It is also to be noted that canned kraut contains less vitamin than does raw kraut.

Loss of Vitamin C During Canning: A number of samples of kraut were followed through the canning operations, beginning with the raw kraut immediately upon removal from the vats. Two of these showed a high content of ascorbic acid in the raw kraut, .40 and .35 mg. per gram (Table 4); a third and a fourth showed about half as much; and a fifth showed only a trace. In the canning process the first step involves a preheating to about 48.9°C.(120°F.) and mixing in a so-called mixing vat. The ascorbic acid content of the kraut after passing through the mixing vat was approximately two-thirds as high as in the kraut before this preheating. This is a continuous process in which ordinarily each sample becomes mixed with the previous samples, but under the experimental conditions as carried out all kraut was removed from the vat and it was then filled with the kraut being studied so as to eliminate the sampling error.

After leaving the mixing vat the kraut is filled into cans which are preheated, filled with brine, closed, and finally processed. During this part of the processing little change is to be noted in the ascorbic acid content.

During subsequent study of other samples of kraut through the fermentation, samples were canned and studied during canning operations. The results of these studies are in agreement with those presented (Table 5).

Vitamin C Content of Kraut During Fermentation: It is obvious, from the results presented, that sauerkraut varies considerably in respect to its vitamin C content even though the different krauts may be in the same stage or age of fermentation. Possibly at some stage during the fermentation, a destruction of vitamin C takes place.

To study this, two five-gallon crocks of kraut were packed and studied during the course of fermentation. The original cabbage contained .34 and .41 mg. of ascorbic acid per gram of kraut, respectively. After 12 days, when the acidities were .98 and .85 per cent, the ascorbic acid content had been reduced to .21 and .14 mg. per gram of juice. For the next 44 days the vitamin content remained at about the same figure. Since the results are so low, apparently these krauts did not represent normal conditions. It has been previously pointed out that samples of raw kraut less than three months old averaged .30 mg. per gram, while a number of samples were above .40 mg. per gram of kraut. These samples were all obtained from large vats holding from 100 to 250 barrels of kraut, and con-

TABLE 5

Vitamin C Content of Kraut During Fermentation, Canning, and Storage

	Tank No. 16				Tank No. 75				Tank No. 41	
	Date—1935	Ascorbic acid in		Acidity as lactic acid	Date—1935	Ascorbic acid in		Acidity as lactic acid	Date—1935	Ascorbic acid in
		Juice	Solids			Juice	Solids			Juice	Solids
		mg. per gm.	*mg. per gm.*	*pct.*		*mg. per gm.*	*mg. per gm.*	*pct.*		*mg. per gm.*	*mg. per gm.*
Cabbage cut	Sept. 12	.03	.41	.....	Oct. 22	.03	.31	.....	Oct. 22	.02	.33
Sauerkraut during fermentation	Sept. 19	.42	.....	1.36	Oct. 29	.40	.....	0.95	Oct. 29	.40	.....
	Sept. 26	.43	.....	1.34	Nov. 5	.36	.....	1.40	Nov. 5	.39	.....
	Oct. 3	.52	.....	1.75	Nov. 12	.35	.....	1.62	Nov. 19	.40	.....
	Oct. 10	.48	.....	1.83	Nov. 19	.38	.....	1.82	Mar. 10	.04	.....
	Oct. 16	.43	.37	.....	Nov. 21	.33 [2]	.31 [2]	1.62	June 25	.02	.03
Mixing tank	Oct. 16	.28	.....	.....	Nov. 21	.26	.....	.....	.....	.....	.....
Filling table	.....	.....	.26	.....	.....	.25	.20	.....	.....	.....	.....
Preheated	.....	.27 [1]	.22 [1]	.....	.....	.24	.17	.....	.....	.....	.....
Before cook	.....	.27	.22	.....	.....	.21	.17	.....	.....	.....	.....
After cook	.....	.27 [1]	.23 [1]	1.40		.25 [1]	.22	.....	.....	.....	.....
Canned kraut	Nov. 16	28 [1]	.26 [1]	1.42	Nov. 22	.24 [1]	.22	1.03	.....	.....	.....
Canned kraut	Dec. 16	.25 [1]	.23 [1]	1.31	Dec. 24	.24	.21	.....	.....	.....	.....
Canned kraut	Apr. 16	.24	.23	.....	Mar. 21	.16	.20	.....	.....	.....	.....

[1] Average of determination on three samples.

[2] Samples from center

Top side —Juice .33, solids .29
Top center —juice .44, solids .37
¼ down —juice ....., solids .35
Bottom center —juice .37, solids .33
Bottom side —juice .02, solids .01.

ditions, particularly in regard to oxygen and carbon dioxide, are different in the larger containers.

Three 250-barrel vats were made available for study one season and two the next season.

The first, tank No. 16 (Table 5), was packed Sept. 12, 1935, and canned 34 days later. The original cabbage in the vat contained .41 mg. of ascorbic acid per gram of solids. Samples of juice were removed from within the vat at weekly intervals by means of an iron pipe and rubber tubing inserted into the kraut. These samples of juice all gave ascorbic acid figures slightly in excess of the original amount in the solids (Table 5). At the end of the 34-day period the solids contained .37 and the liquid .34 mg. per gram. This kraut had fermented rapidly, attaining an acidity of 1.75 per cent as lactic acid in 21 days. The kraut was of excellent quality.

The second vat, No. 75, was filled on October 22 and canned on November 21. The original cabbage contained .31 mg. per gram and the juice during fermentation, from .33 to .40 mg. of ascorbic acid per gram of solids. The final solids also contained .31 mg. per gram. This kraut also fermented rapidly, attaining an acidity of 1.62 per cent in 21 days, and was of excellent quality.

Kraut samples from both tanks, No. 16 and No. 75, were studied during the canning process. The ascorbic acid content was reduced to .28 and .26 mg. per gram respectively, after passing through the mixing tank. This content was reduced only slightly during the canning process, and even in the canned kraut held for six months little reduction was noted.

The third vat of kraut, No. 41, was also packed on October 22. The cabbage contained .33 mg. per gram of solids. Like the other two, the kraut fermented rapidly and the juice showed an increase in ascorbic acid over the original solids. The original plan was to leave this tank until spring but, through oversight, juice was removed from it some time in February and its removal was not reported until March. The kraut was immediately sampled and was found to show only .04 mg. of ascorbic acid per gram of juice. Samples taken from the vat when canned in June all yielded low results, .01 to .03 mg. per gram. Unfortunately the rapidity of destruction of ascorbic acid after draining was not determined. Furthermore, samples taken from other vats which had been drained similarly did not show this marked destruction of ascorbic acid. Apparently destruction of the vitamin was due in large part to the loss of carbon dioxide and the introduction of air during the draining of the vat.

The following season two vats of kraut were packed, No. 61 and No. 62 (Figs. 1 and 2), and studied in the same manner. Samples

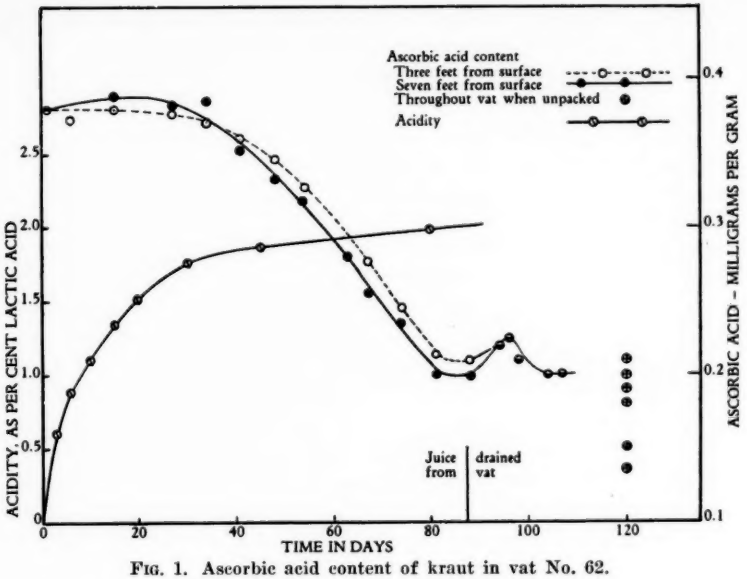

FIG. 1. Ascorbic acid content of kraut in vat No. 62.

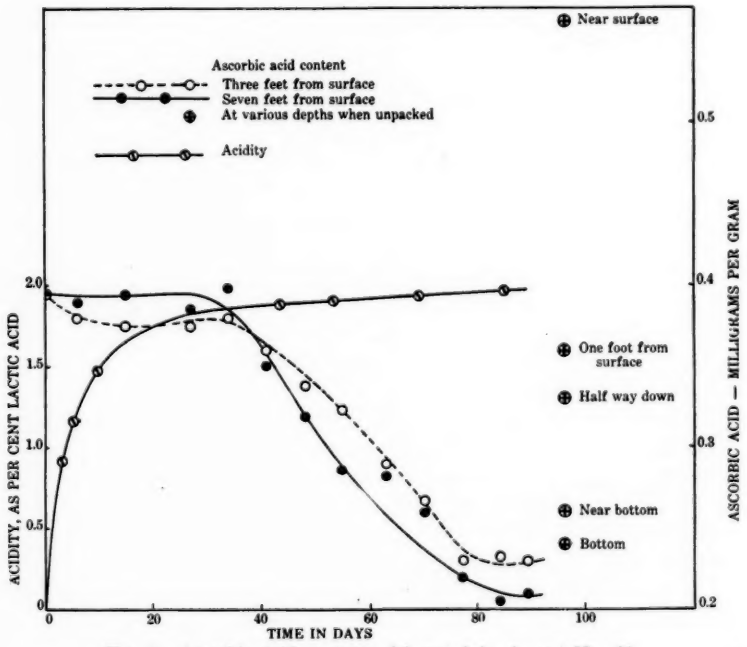

FIG. 2. Ascorbic acid content of kraut juice in vat No. 61.

of juice and kraut were treated with hydrogen sulfide to reduce the dehydro-ascorbic acid. In no case was any considerable amount of reversibly oxidized ascorbic acid found. In both vats the kraut fermented rapidly, an acidity of more than 1.7 per cent being attained in less than 15 and 25 days. The content of ascorbic acid remained practically stationary (Figs. 1 and 2) for another 20 days or more. Samples obtained on the 41st day showed a decrease in vitamin C and this decrease continued gradually.

In vat No. 62 a level of around .20 mg. per gram seemed to have been attained at about the 80th day, holding at the level until canned

TABLE 6

Vitamin C Content of Kraut From Vat No. 62

Source of sample	Ascorbic acid	
	Juice	Solids
	mg. per gm.	*mg. per gm.*
Near surface—		
Edge of vat	.21	.20
Two feet from edge	.21	.21
Center of vat	.22	.22
One foot from surface—		
Edge of vat	.19	.19
Two feet from edge	.19	.19
Center of vat	.18	.18
Six feet from surface—		
Edge of vat	.19	.18
Two feet from edge	.21	.18
Center of vat	.20	.19
Near bottom—		
Edge of vat	.15	.15
Two feet from edge	.16	.14
Center of vat	.15	.15

25 days later, or when 3½ months old. The free juice at the bottom of the vat was drained off 21 days before canning but this did not cause a change in the ascorbic acid content. While the kraut was being removed for canning, samples were obtained from the top, one foot from the top, halfway, and at bottom levels of the tank. At each level samples were obtained at the center, at the side, and halfway between. These samples varied in ascorbic acid content from .14 to .22 mg. per gram and averaged .190 mg. per gram (Table 6). The average increase in ascorbic acid content following reduction with hydrogen sulfide was .02 mg. per gram, indicating the presence of a small but definite amount of dehyro-ascorbic acid.

The kraut in vat No. 61 fermented more rapidly and showed the same decrease in ascorbic acid content beginning after the kraut was 40 days old (Fig. 2). The kraut was canned when 89 days old. No juice had been drained in the meantime. Samples were again obtained from throughout the vat, as in vat No. 62, but in contrast a decided variation was obtained in vitamin C content. The three samples obtained from the top contained .50, .59, and .58 mg. per gram of juice and .45, .47, and .49 mg. per gram of solids (Table 7). These

TABLE 7

Vitamin C Content of Kraut From Vat No. 61

Source of sample	Ascorbic acid	
	Juice	Solids
	mg. per gm.	*mg. per gm.*
Near surface—		
Edge of vat	.50	.45
Two feet from edge	.59	.47
Center of vat	.58	.49
One foot from surface—		
Edge of vat	.40	.38
Two feet from edge	.36	.34
Center of vat	.33	.33
Halfway down—		
Edge of vat	.35	.34
Two feet from edge	.33	.32
Center of vat	.31	.31
Near bottom—		
Edge of vat	.27	.24
Two feet from edge	.26	.22
Center of vat	.25	.21
Bottom juice	.24	.....

results are higher than those obtained in the original cabbage. This may have been due to variation in the original cabbage but it seems more logical to assume that it is at least partially a drainage effect. When kraut is first made, a considerable amount of juice is formed, a large part of which drains through to the bottom. This juice ordinarily contains little ascorbic acid so that the solids, particularly near the top, would show a concentration of the vitamin.

The samples obtained throughout the tank all showed ascorbic acid contents in excess of the samples obtained from pipe samples. These averaged .350 before and .368 mg. per gram at the time of canning, the curves showing a gradual decrease in vitamin C. These results cannot be explained merely on the basis of differences owing

to drainage. It would seem more logical to assume that the kraut in the vicinity of the region where pipe samples were taken was affected by the sampling procedure and therefore the vitamin C was destroyed more rapidly than in other parts of the vat. This probably was due to liberation of carbon dioxide and subsequent introduction of air.

Kraut canned from vat No. 61 showed practically the same reduction in vitamin C as did previously canned krauts similarly examined. The fresh kraut contained .28 mg. per gram in the juice and .24 mg. per gram in the solids. This was reduced to .18 mg. per gram in the

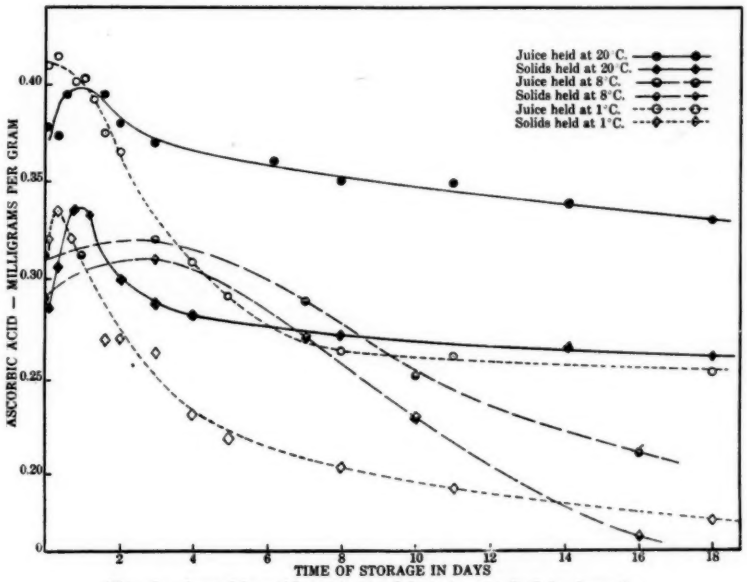

FIG. 3. Ascorbic acid content of kraut repacked in barrels.

juice as well as in the solids. The final canned kraut contained .18 mg. per gram.

Effect of Repacking in Barrels: Two five-gallon kegs were packed with kraut from near the top of vat No. 61 while a ten-gallon keg was filled from near the center of the vat. The first of these was stored at 20°C.(68°F.), the second at 1°C.(33.8°F.), and the third at 8°C.(46.4°F.) for 18 days. Ascorbic acid determinations were made at frequent intervals during the first few days but less frequently during the latter part of the storage period. The solids as well as liquids were examined. During this period an abrupt increase was noted after packing, followed by a gradual decrease (Fig. 3).

This decrease was more rapid in the kraut stored at 1°C. and 8°C. than in that stored at 20°C., the former dropping from an original .41 mg. in juice and .31 mg. in solids to .25 and .18, while that stored at 20°C. dropped from .38 mg. in the juice and .28 mg. in the solids to .33 and .26 during the 18-day period. These results appear to be explainable only on the basis that at the higher temperature continued fermentation and production of carbon dioxide protected the vitamin from atmospheric oxidation. A greater loss of vitamin C occurs at the lower storage temperature because the fermentation is very slow, practically no carbon dioxide is formed, and the vitamin C is exposed to the air.

DISCUSSION

Throughout the series of experiments a loss of from one-third to one-half of the vitamin C in the kraut has been shown, not only in canning kraut but also in holding it in the vat after fermentation is complete and in repacking it in barrels. During prolonged storage this loss may be even greater. In the few cases tested, in which juice was drained from the vat some time previous to unpacking, a considerable loss of ascorbic acid was noted. During fermentation a considerable amount of carbon dioxide gas is produced. Preuss, Peterson, and Fred (1928) have shown that the oxygen present is almost entirely replaced by carbon dioxide during the first few days. It has been observed further that the carbon dioxide dissolved in newly fermented kraut juice is slowly lost upon aging. It is apparent that the loss of vitamin C begins only after the fermentation process, as measured by the formation of acid, has been completed and the production of carbon dioxide has practically ceased (Figs. 1 and 2).

The foregoing results seem to indicate that the reduction of vitamin C is due more to a loss of the protective atmosphere of carbon dioxide than to any other factor.

CONCLUSIONS

The vitamin C content of sauerkraut has been studied in relation to the original cabbage, changes taking place during fermentation, during storage, during canning, and after repacking in barrels.

The ascorbic acid (vitamin C) content of the various samples was determined by titration with 2,6 dichlorophenolindophenol.

Raw kraut obtained during and immediately after fermentation in the large, commercial-size vats contains approximately the same amount of vitamin C as the original cabbage. During storage in the vats after fermentation the content of vitamin C slowly decreases. In kraut fermented in smaller containers the loss of vitamin C is more rapid.

Canned kraut contains less vitamin C than raw kraut. The lowering of the vitamin C content takes place primarily in the mixing, preheating vat before the kraut is put into cans. Little decrease was noted during further processing or during storage.

Kraut repacked in barrels loses vitamin C. The loss was more rapid in barrels stored at low temperature, 1°C., than at higher temperature, 20°C., where fermentation could continue.

The loss of vitamin C in kraut seems to be associated with the loss of carbon dioxide after fermentation is complete.

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SOURCES OF URONIC ACID IN THE APPLE

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Previous work in this laboratory has indicated that food sources of uronic acids are important. It is believed that the uronic acid needed for the detoxication mechanism may be obtained in this way under certain conditions. Further, there seems to be a connection between vitamin A metabolism and uronic acid metabolism, according to Manville (1937). It seemed necessary, therefore, to discover what foods are rich in uronic acid, and further, how the uronic acid sources are distributed in any particular food.

The Winesap apple was found to have a high uronic acid content. In preliminary work two types of determinations were made: first, the amount of CO_2 yielded by whole apple was determined by Link's method, presented by Dickson, Otterson, and Link (1930); second, pectin was determined by the calcium pectate method, used by Nanji and Norman (1928). It was noted that the pectin content alone could by no means account for the CO_2 values obtained. This discrepancy was even more striking in similar work on pears (Anjou and Nelis). The data of this paper were obtained in an attempt to determine the sources of CO_2 in the apple.

METHODS OF ANALYSIS

In order to find the true sources of uronic acid in the fresh apple it was converted to a dehydrated, pulverized form. Fractionation methods were then used to isolate the different components into two or more arbitrary solubility groups and each was analyzed for its uronic acid content.

Extraction of Winesap Powder With 95-Per Cent Ethanol: About five grams of finely powdered Winesap were thoroughly desiccated over H_2SO_4 *in vacuo*. The dried sample was placed in an extraction thimble which had been previously extracted with boiling 95-per cent ethanol. Owing to the highly hygroscopic nature of the apple powder, each sample was dried to a constant weight. The weighed sample was extracted in a Soxhlet extractor with 150 c.c. of 95-per cent ethanol. At the end of 48 hours practically all alcohol-soluble compounds were extracted from the powder. The residue in the thimble was first dried in an electric oven at 70°C.(158°F.) for several hours and then over H_2SO_4 *in vacuo*. The alcohol-soluble portion, which

contains sugars, must be dried at a lower temperature to prevent charring. For convenience, the volume of alcohol extract was measured accurately, warmed, stirred thoroughly, and 10-c.c. portions of solution were pipetted into a small weighed beaker. Ten cubic centimeters of water were added to each sample; the samples were dried in an oven at 60°C.(140°F.) for two days and then over H_2SO_4 *in vacuo* for several days.

Extraction of Alcohol-Insoluble Portion With Water: The alcohol-insoluble portion was further extracted with 200 c.c. of water for a period of 48 hours to separate water-insoluble cellulose, hemicelluloses, and lignin from the water-soluble portion which contained pectin, other colloidal substances, and salts. The residue and extract then were both dried at 100°C.(212°F.) in a desiccator. The results are shown (Table 1).

TABLE 1
Fractions of Apple Powder

Alcohol insoluble	Alcohol soluble	Water insoluble	Water soluble
pct.	*pct.*	*pct.*	*pct.*
17.1	82.9	9.3	8.6
18.0	82.0	9.9	7.1
16.4	83.5	9.2	8.8
17.5	82.5	.....	.....
17.2	82.8	.....	.....
17.0	83.0	.....	.....

Uronic Acid Determinations: Link's method was used to determine the uronic acid content of each component. Weighed samples were mixed with 100 c.c. of 12-per cent HCl in a 250-c.c. extraction flask with a ground-glass joint and a side arm. The flask was connected to a Friedrich's condenser and placed in an oil bath. The mixture was then preheated at 75°C.(167°F.) for an hour, while a slow stream of CO_2-free air was passed through the side arm to sweep out residual carbon dioxide. At the end of an hour the temperature of the oil bath was raised to 140°C.(284°F.) and maintained at that temperature throughout the determination. The CO_2 evolved from the sample was first washed with a 10-per cent silver nitrate solution and then absorbed by .2 N $Ba(OH)_2$ in the absorbing tower. At the end of six hours, excess $Ba(OH)_2$ was titrated with .1 N HCl to phenophthalein end point. Six hours of heating was chosen because most of the uronic acid was decomposed in that period, although additional small quantities of CO_2 were evolved after 12 hours of heating. All values were calculated as uronic anhydride (Tables 2A and 2B).

Slight differences in the materials used and differences in the rate of extraction, even when utmost care was taken to control physi-

TABLE 2A

Uronic Acid Values

Fraction	Per cent of apple powder and fractions	Calculated as per cent of apple powder
Apple powder (Winesap)	5.60	.....
	5.93	.....
	5.40	.....
	5.66	.....
Alcohol-insoluble portion	15.67	2.71
	15.52	2.81
	15.81	2.68
	15.84	2.69
	15.85	2.69
	15.83	2.69
Alcohol-soluble (apparent)	3.00	2.48
	3.60	2.98
	2.55	2.11
	4.00	3.31
	3.61	2.98
	3.46	2.86
Alcohol insoluble-water insoluble	8.40	0.794
	8.18	0.767
	7.61	0.715
	7.52	0.744
	7.93	0.704
	8.07	0.759
Alcohol insoluble-water soluble	19.76	1.695
	17.89	1.535
	21.76	2.040
	19.24	1.650
	19.56	1.678
	19.84	1.710

TABLE 2B

Corrected Uronic Acid Values

Fraction	Calculated as per cent of apple powder	Washed-yeast procedure [1]
Alcohol soluble	.667	.....
	.800	.....
	.567	.....
	.879	.....
	.800	.777
	.769	.694

[1] Somogyi (1928).

cal conditions, made exact checks impossible. One may notice about a 10-per cent loss in the uronic anhydride values of the alcohol-insoluble portion by further extraction with water. This loss may be caused by the adsorption of pectin on extraction thimbles.

Since it is difficult to control physical conditions in the extractions, several different methods of analysis were chosen instead of attempting to check the values by increasing the number of determinations.

Extraction of Winesap Powder With Water: Five-gram samples of Winesap powder were extracted for 48 hours in a Soxhlet extractor with 200 c.c. of distilled water. The residue was dried in the usual manner and weighed. Ten-cubic centimeter portions of aqueous extract were first oven-dried at 60°C.(140°F.), then over H_2SO_4 for two days. The data are given (Table 3).

TABLE 3

Extraction of Apple Powder With Water

Water insoluble	Water soluble
pct.	*pct.*
8.0	81.1
6.2	93.8
8.4	91.6
8.8	91.2

Completely dried samples of residue from the aqueous extracts were so hard that overnight soaking was necessary before uronic acid values could be run.

Elimination of Sugar: The water-soluble portion contains a complex mixture. Most of the substances which yield carbon dioxide by treatment with 12-per cent HCl are extracted by water. Further procedure eliminated interfering sugars from the extract. The first method utilized the Somogyi (1928) washed-yeast procedure. The dilute-water extract was mixed with washed yeast and incubated at 37°C.(98.6°F.) in a water bath. Small samples were pipetted out from time to time and the sugars titrated by the Shaffer-Hartman method. As soon as the titration indicated complete fermentation of reducing sugars, the liquid was decanted off. The yeast mixture was filtered and the filtrate combined with the decanted liquid. The combined liquid was set aside for several hours and decanted again to eliminate yeast suspensions. This liquid was slowly concentrated to a volume of 150 c.c., enough concentrated HCl was added to make a 12-per cent solution, and the carbon dioxide value determined (Table 2B).

Alcohol extraction showed definitely that the substances contributing most of the CO_2 are precipitated with ethanol. The aqueous

extract from five grams of apple powder was concentrated to a volume of 25 c.c. and precipitated with 90 c.c. of 95-per cent ethanol, acidulated with five drops of concentrated HCl. The precipitate—containing most of the pectin—was filtered off, dried in an electric oven, and its CO_2 value determined (Table 4).

TABLE 4

Uronic Acid Values

Water insoluble		Water soluble	
Sample	Calculated as per cent of apple powder	Sample	Calculated as per cent of apple powder
pct.		*pct.*	
		(CO_2 evolved from sugars not subtracted)	
3.59	.22	4.88	4.6
4.00	.24	4.72	4.4
		4.90	4.6
		5.03	4.7
8.28	.71		
6.93	.61	4.50	4.1
		(Alcohol precipitate)	
		3.59	3.3
		(Fermented with Somogyi washed yeast)	
8.36	.83	3.27	3.0
8.36	.83	3.31	3.0

In the second method, dialysis was employed to eliminate sugar. Since collodion bags could be made easily and since sugars can be dialyzed out while retaining colloidal substances, it would seem that their use would eliminate sugar in the apple powder. (It contains nearly 70 per cent by weight of sugar.) The values obtained by this method give a sum total of uronic acid in the form of colloidal substances in the apple powder and amounted to 3.16 per cent calculated as percentage of apple powder.

Calcium Pectate Method: Pectin in the apple powder was determined as calcium pectate. Carbon dioxide values were determined both for calcium pectate and the residue. The uronic acid values are shown (Table 5).

TABLE 5

Uronic Acid Values

Residue calculated as per cent of apple powder	Calcium pectate calculated as per cent of apple powder
.64	2.05
.68	2.06
.76	1.80
.64	2.13

DISCUSSION

From the data it will be seen that the distribution of sources of CO_2 in apple powder is about as follows:

Pectin	2.1	37.5%
Sugars	2.0	35.7%
Hemicelluloses	0.7	12.5%
Unknown in alcohol-soluble portion	0.8	14.3%
CO_2 value of apple powder calculated as uronic anhydride	5.6	100.0%

It will be noted that a large fraction is contributed by sugars. We feel that in the past this has been overlooked in many uronic acid determinations. We tested the acids of the apple, cellulose, lignin, and starch as extraneous sources of CO_2 and found them to be insignificant.

Pectin is undoubtedly the major source of uronic acid although other sources combine to contribute, in most cases, nearly as much. The constituents of the alcohol-soluble fraction after the removal of the sugars may consist of lower polymers of uronic acids. Color tests for free uronic acids are negative. In the fresh fruit the ascorbic acid would undoubtedly contribute some small amount of CO_2.

Interested in knowing the approximate percentages of hemicellulose, cellulose, and lignin in the apple, we determined them by the method of Williams and Olmstead (1936). Indicative values are as follows:

Lignin	2.5% of apple powder	Uronic anhydride absent
Cellulose	3.0% of apple powder	Uronic anhydride absent
Hemicellulose	2.5% of apple powder	Uronic anhydride about 8%
Pectin	5.0% of apple powder	Uronic anhydride about 60%

From the nutritional standpoint the question of availability is still to be considered. Pure pectin seems not to give the same results as apple powder. This may be due to small amounts of readily available uronic acid in the powder or possibly to the effect, chemical or physical, of the apple matrix.

CONCLUSIONS

1. Sources of CO_2 in the apple have been determined.

2. The percentage distribution of unavailable carbohydrate is indicated.

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SALT CONTENT OF CURED HAM[1,2]

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The preservative action of salt has always been of primary importance in relation to the curing of ham. Recent work by Jones (1937a, b) indicates that a further function of salt lies in its selective action in addition to its bactericidal effect. Thus nitrate-reducing organisms function in high concentrations of sodium chloride which are detrimental to microörganisms having a deleterious effect on the meat.

Preservation alone does not fulfill all of the requirements of a successful cure. The further requirement for ham is for a palatable product that is not oversalted.

The present investigation was instigated because there was little definite information available to those who wish to produce a home-cured product of the best quality as judged from the viewpoint of preservation and palatability. A study was made of different curing methods from the standpoints of practicability of the method and of quality of the resulting product. This report is limited to a consideration of the salt content of hams cured by three procedures, and to a comparison of these hams with standard commercial hams obtained on the local market.

EXPERIMENTAL PROCEDURE

Forty hams were used. Of these, eight were purchased at a local butcher shop and represented first-quality products produced by nationally known meat packers. The salt content of these hams was determined for purposes of information and for comparison with our own experimentally cured hams.

Twelve hams were dry-cured under definite experimental conditions. The curing agent was composed of eight pounds of salt, three pounds of sugar, and three ounces of saltpeter (KNO_3). It was applied at the rate of one ounce per pound of meat. Usually the application of the curing agent involved rubbing the mixture into the hams in two or three portions at three-day intervals, applying as much each time to the meat as it would take up, until the required amount was used.

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[1] Presented before the division of agricultural and food chemistry at the Rochester meeting of the American Chemical Society, September 6 to 10, 1937.

[2] Authorized for publication on January 7, 1938, as paper No. 820 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

Eleven hams were cured experimentally in a 75-degree (salometer) sweet pickle which was made up of the same mixture as was used for dry curing but which was dissolved in five gallons of water. The use of four gallons of water produced an 85-degree pickle which was used for the curing of nine hams.

These hams were sampled and analyzed by the methods given by Miller and Ziegler (1936) and Ziegler and Miller (1938). Five subsamples were taken from each ham (Fig. 1). Only the salt contents of the subsamples from the lean portion of the ham (No. 2, 3, 4, 5) are given in this paper; those for subsample No. 1 are omitted because this subsample usually consisted largely of fat and did not absorb salt to the same degree as did the lean tissue.

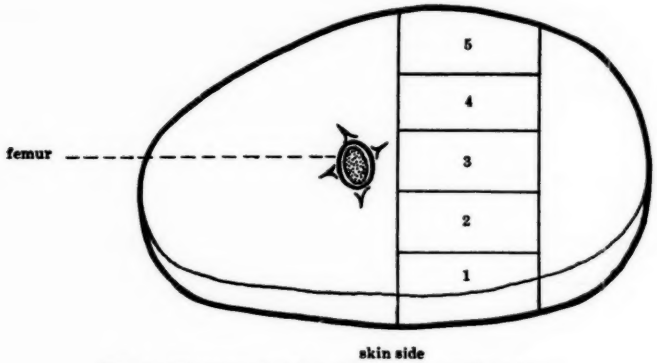

FIG. 1. Diagram showing method of sampling ham.

The method of sampling gives information as to the condition of vital portions of the ham as considered from the viewpoint of effectiveness of the cure with respect to salt penetration. The No. 3 subsample is from the center of the ham, a region next to the bone where spoilage is very likely to occur in the case of incomplete curing. The salt content of this subsample, when considered in relation to the salt content of the adjacent subsamples, gives a good idea of the extent to which salt has penetrated the ham.

SALT CONTENT OF COMMERCIAL BRANDS OF HAM

In order to ascertain what constitutes a good-quality, first-grade, commercial brand of ham as considered from its content of salt, a number of nationally known brands of ham were studied. No information is available concerning the manner in which these hams were cured but they are judged to be representative of first-quality prod-

uets. These hams were sampled and the samples analyzed by the methods given above.

The data for eight such representative hams (Table 1) show that in all cases no less than 13.5 per cent of salt was present in the dry matter of the outer lean portions of these hams (Subsamples 4 and 5), and in some cases over 18 per cent was present.

Subsample No. 2, from next to the fat or skin side of the ham, and No. 3, from the center, did not contain as much salt as Subsamples 4 and 5 in spite of a possibility that the centers of these hams were pumped with a strong pickle as a part of the cure, a common commercial practice.

TABLE 1

Salt Content of Commercial Hams

Ham No.	Brand	Mean NaCl content [1] of center slice	NaCl content [1]			
			Subsample No. 2	Subsample No. 3	Subsample No. 4	Subsample No. 5
		pct.	*pct.*	*pct.*	*pct.*	*pct.*
1	A	11.63	11.44	7.45	13.68	13.92
2	A	12.91	13.67	9.99	14.43	13.54
3	A	12.51	12.16	7.91	14.50	15.46
4	B	15.75	16.53	12.87	18.06	15.52
5	B	16.35	15.88	12.63	18.36	18.54
6	B	17.42	17.73	15.56	18.49	17.91
7	C	13.97	11.09	11.22	15.41	18.14
8	C	16.18	18.14	12.21	17.65	16.73
Mean	....	14.59 ± .51	14.58	11.23	16.32	16.22

[1] NaCl in dry matter.

The mean salt content of the entire center slice, as computed from the four subsamples, ranged from 11.63 per cent to 17.42 per cent, the mean for the eight hams being 14.59 per cent.

It would appear, therefore, as judged from these hams, that 11 per cent of salt is sufficient for purposes of preservation in the center of the ham next to the bone, where spoilage is most likely to occur. It is probable that 11 per cent of salt is also sufficient to provide a considerable factor of safety in so far as the keeping quality of ham is concerned. Such a factor of safety is perhaps desirable for a commercial product which may be subjected to more or less variable conditions and hazards during storage and distribution before the meat finally reaches the consumer.

It is to be noted that, while the salt contents of all of the eight hams are of the same general order of magnitude, those of Brand A

TABLE 2

Salt Content of Dry-Cured Hams

Ham No.	Curing period	Aging period	Curing temperature	Fresh weight		Thickness	Mean NaCl content [1] of center slice	NaCl content [1]			
								Subsample No. 2	Subsample No. 3	Subsample No. 4	Subsample No. 5
	days	*days*		*lb.*	*oz.*	*in.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*
1	28	30	1.1 to 2.8°C. (34 to 37°F.)	15	12	5½	9.63	8.50	8.84	9.29	11.87
2	35	30	1.1 to 2.8°C. (34 to 37°F.)	16	10	5½	9.19	8.50	8.97	8.46	10.82
3	42	30	1.1 to 2.8°C. (34 to 37°F.)	16	6	5¼	11.27	9.97	10.73	11.50	12.89
4	26	30	1.1 to 2.8°C. (34 to 37°F.)	14	1	5¼	12.48	13.13	10.30	12.50	14.00
5	40	30	1.1 to 2.8°C. (34 to 37°F.)	15	13	5½	10.87	11.25	9.69	10.71	11.83
6	28	30	Uncontrolled	14	7	4½	7.95	6.08	7.89	8.17	9.67
7	35	30	Uncontrolled	15	11	5	8.51	7.75	7.80	8.44	10.06
8	42	30	Uncontrolled	16	3	5	8.04	7.09	7.90	8.29	8.86
9	30	30	1.1 to 2.8°C. (34 to 37°F.)	14	12	4¾	11.24	10.02	8.70	11.23	15.00
10	35	30	1.1 to 2.8°C. (34 to 37°F.)	14	6	4¾	10.12	7.61	7.20	10.57	15.10
11	31	30	Uncontrolled	12	6	4¾	13.52	13.31	10.86	15.87	14.02
12	33	30	Uncontrolled	13	8	4¾	13.25	12.52	11.06	14.31	15.09
Mean	....	....	.....	....	....	.....	10.51 ± .38	9.64	9.16	10.80	12.44

[1] NaCl in dry matter.

contain somewhat less salt than those of the other two brands, indicating a somewhat milder cure for this brand.

SALT CONTENT OF DRY-CURED HAMS

Preliminary work by the authors has shown that a satisfactory dry-cure was obtained with hams when a curing formula consisting of eight pounds of salt, three pounds of sugar, and three ounces of saltpeter (KNO_3) was applied at the rate of one ounce per pound of meat. This formula or some modification of it is considered as a standard curing mixture and is recommended rather extensively for agricultural use.

Some idea of the salt content of the final product cured by this formula can be obtained by computation. Applying one ounce per pound of the 8-3-3 curing agent to a 12-pound ham containing 22 ounces of bone and 45 per cent of dry matter would result in about 11 per cent of salt in the dry matter of the ham minus the bone. However, there is some loss of salt from the ham through the brine that drains from it during dry-curing, hence 11 per cent of salt represents the maximum amount which could be present.

Data for 12 hams dry-cured by the 8-3-3 formula show that the average amount of salt in the dry matter of the center slices from which the subsamples were taken is $10.51 \pm .38$ per cent (Table 2). This indicates that the 8-3-3 formula gives a somewhat milder cure with respect to salt than that of the commercial hams. The average salt content of the subsample taken from the tissue next to the bone (No. 3) is 9.16 per cent, which is lower by approximately two per cent than the salt content of this subsample in the commercial hams. For Subsamples 4 and 5 differences are even greater, the dry-cured hams containing less salt by 5.52 per cent in subsample No. 4 and 3.78 per cent in No. 5. It is apparent, therefore, that the cure used for these hams gives a product which contains somewhat less salt than is found in the commercial hams used for comparison.

SALT CONTENT OF BRINE-CURED HAMS

Data for 11 hams cured in a 75-degree sweet pickle, made up as previously stated, show that the amount of salt, $10.15 \pm .48$ per cent, representing the average for the center slices for all the hams is not significantly different from that of the dry-cured hams (Table 3). As compared with the dry-cured hams, however, there is slightly less salt on the average in the No. 3 center subsample and somewhat more in the outside No. 4 and No. 5 subsamples. This indicates that the 75-degree pickle does not penetrate and strike into the center of the hams as rapidly as the salt does in the dry-curing. Nevertheless, the

TABLE 3

Salt Content of Hams Cured in 75-Degree Sweet Pickle

Ham No.	Curing period	Aging period	Curing temperature	Fresh weight		Thickness	Mean NaCl content [1] of center slice	NaCl content [1]			
								Subsample No. 2	Subsample No. 3	Subsample No. 4	Subsample No. 5
	days	*days*		*lb.*	*oz.*	*in.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*
13	40	....	1.1 to 2.8°C. (34 to 37°F.)	15	6	4 5/7	7.95	4.68	4.73	6.03	16.36
14	30	30	1.1 to 2.8°C. (34 to 37°F.)	14	2	4 3/7	7.45	5.51	5.16	8.93	10.20
15	35	30	Uncontrolled	11	5	4 3/7	6.35	6.70	4.43	5.98	8.30
16	45	30	Uncontrolled	15	5	4 6/7	11.97	10.54	8.02	14.44	14.86
17	45	30	Uncontrolled	14	10	4 4/7	10.49	9.49	8.27	11.28	12.92
18	34	30	1.1 to 2.8°C. (34 to 37°F.)	12	5	4 5/7	13.04	12.19	9.74	15.50	14.73
19	50	30	1.1 to 2.8°C. (34 to 37°F.)	13	3	4 5/7	12.89	8.86	10.57	13.84	18.30
20	30	30	1.1 to 2.8°C. (34 to 37°F.)	10	0	4 2/7	12.87	13.78	9.85	12.72	15.13
21	30	30	1.1 to 2.8°C. (34 to 37°F.)	11	5	4 3/7	10.17	10.13	9.17	11.08	11.31
22	35	30	1.1 to 2.8°C. (34 to 37°F.)	10	13	4 3/7	8.43	6.08	7.65	10.25	9.72
23	34	30	1.1 to 2.8°C. (34 to 37°F.)	12	8	4 4/7	10.04	8.69	6.99	12.80	11.76
Mean	....	....	.....	....	....	.....	10.15 ± .48	8.79	7.69	11.17	13.05

[1] NaCl in dry matter.

saltiness of the final product is about the same as that of the dry-cured hams and is less than that of the commercial hams.

The data for the 75-degree sweet pickle hams, however, are more variable than those previously considered. This is true for samples within the individual hams as well as for samples from different hams cured by this method. While the agreement leaves much to be desired from an experimental viewpoint, the data obtained may indicate that the 75-degree sweet-pickle cure is somewhat slower in action than the dry-cure. The additional possibility remains that some of this variation may also be due to the hams themselves, uniformity of which is difficult to attain, owing to such factors as variable composition of the tissue and individual differences existing in the hogs from which the hams were taken.

The data for hams cured in an 85-degree sweet pickle indicate that the mean salt content of the center slice for all of the hams is not significantly different than that for the dry-cured and 75-degree pickle hams (Table 4). A better penetration and equalization of salt throughout the various subsamples is indicated by the mean salt contents which range from 9.61 per cent for Subsample 2 to 10.89 for Subsample 4. The salt content of the outside section (Subsample 5) of the hams is lower on the average by 5.77 per cent than that in the commercial hams. A similar relationship exists for all of the other subsamples except No. 3, the center subsample, in the case of which the commercial hams are higher in salt content by 1.33 per cent.

Somewhat better uniformity is apparent for all of the hams than was the case with the 75-degree cure, a fact perhaps due to the better penetration of the salt from the 85-degree brine. A further fact which may contribute to the uniformity of the data for this cure is that in some cases hams of this cure were subjected to a somewhat longer aging period than in the other cases during which there was more complete equalization of the salt throughout the meat tissue.

DISCUSSION OF RESULTS

A summary of the data with respect to the salt content of all of the hams used in this study (Table 5) shows that the average salt content of the entire center slice for all three groups of experimentally cured hams was about the same, and was somewhat lower than that of the commercial hams. Also, with respect to the various subsamples, on the average, none of the experimental hams was as salty as the commercial hams. There was no evidence that the lower salt content of these hams impaired their keeping quality. This does not mean that a somewhat higher salt content would not be desirable from the

TABLE 4

Salt Content of Hams Cured in 85-Degree Sweet Pickle

Ham No.	Curing period	Aging period	Curing temperature	Fresh weight		Thickness	Mean NaCl content [1] of center slice	NaCl content [1]			
								Subsample No. 2	Subsample No. 3	Subsample No. 4	Subsample No. 5
	days	*days*		*lb.*	*oz.*	*in.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*
24	32	30	1.1 to 2.8°C. (34 to 37°F.)	11	0	4 2/7	10.00	9.46	9.30	10.97	10.28
25	28	61	Uncontrolled	14	14	5 1/2	8.02	7.32	8.99	8.24	7.52
26	35	54	Uncontrolled	15	5	5 1/2	9.46	8.30	8.90	10.21	10.41
27	42	61	Uncontrolled	15	14	5 1/2	11.15	9.76	13.08	11.12	10.64
28	28	30	1.1 to 2.8°C. (34 to 37°F.)	13	7	5	9.58	9.30	9.33	9.76	9.92
29	42	60	1.1 to 2.8°C. (34 to 37°F.)	16	10	5 1/2	11.29	11.45	12.23	10.82	10.64
30	35	60	1.1 to 2.8°C. (34 to 37°F.)	14	3	5	9.63	11.32	10.61	9.10	7.49
31	35	30	1.1 to 2.8°C. (34 to 37°F.)	12	10	4 1/2	13.29	10.39	10.02	15.91	16.84
32	28	30	1.1 to 2.8°C. (34 to 37°F.)	10	3	3 5/7	9.48	9.16	6.63	11.84	10.29
Mean	....	....	.....	....	....	.....	10.21 ± .34	9.61	9.90	10.89	10.45

[1] NaCl in dry matter.

standpoint of keeping quality under uncertain storage and handling conditions, but the keeping quality of these hams is judged to be satisfactory for farm conditions.

It will be observed that some of the hams were cured under controlled temperature conditions at 1.1 to 2.8°C.(34 to 37°F.) and that some were cured under uncontrolled conditions which were approximately 8.3°C.(47°F.) in the winter and 12.8°C.(55°F.) in the spring and fall. No differentiation has been made in this study between hams cured under these two different temperature conditions because they prevailed only during the curing period proper, and all of the hams were subsequently exposed to approximately the same environmental temperatures during the aging or holding period which followed the initial period in cure.

TABLE 5

Average Salt Content[1] of Center Slice of Hams

Number of hams	Kind of ham	Average salt content[1]				
		Entire center slice	Subsample No. 2	Subsample No. 3	Subsample No. 4	Subsample No. 5
		pct.	*pct.*	*pct.*	*pct.*	*pct.*
8	Commercial (purchased locally)...	14.59 ± .51	14.58	11.23	16.32	16.22
11	Sweet pickle (75°) cure.....	10.15 ± .48	8.79	7.69	11.17	13.05
9	Sweet pickle (85°) cure.....	10.21 ± .34	9.61	9.90	10.89	10.45
12	Dry-cured.....	10.51 ± .38	9.64	9.16	10.80	12.44

[1] NaCl in dry matter.

Also, heretofore no mention has been made concerning the length of curing periods. The number of days in cure is given in the tables and will be found to be different for different hams. These curing periods were established for purposes other than the immediate object of this paper, and the chief justification for use of the data presented here is that the initial curing periods were followed by the above-mentioned aging or seasoning period which was at least 30 days long. During this period all of the hams were subjected to the same environmental conditions and were smoked. It was during this period that the cure was really completed. No further absorption of salt is possible during this time, but that which already has been absorbed, and is present to a large extent in most cases in the outer portions of the ham, continues to penetrate the tissue and to strike into the center of the ham, eventually becoming more or less evenly distributed throughout the whole piece. This process of sale equalization has previously been reported on by Ziegler and Miller (1938).

This latter consideration does not ignore the fact that in brine-curing more salt is taken up by the meat upon longer contact with

the curing agent. However, there has been some misconception concerning the length of the curing period as applied to dry-curing. In this case, inasmuch as the curing agent is applied directly to the meat in definite amounts and is all absorbed within a relatively few days, the proper conception of the curing period is a length of time sufficient for the salt to strike into the meat and become distributed in concentrations sufficient to prevent spoilage. A prolonged curing period, in the case of dry-curing, will not affect the amount of salt ultimately present in the meat, this having been determined by the amount of curing agent that was applied.

In the case of the brine-cure a different situation exists and the length of the curing period directly influences the amount of salt taken up by the meat. The authors have thought it more logical to consider the length of the curing period in relation to the thickness of the cuts to be cured rather than in relation to their weight. This viewpoint is arrived at from a consideration that the efficacy of salt in curing is due to its penetration of the meat, a process most logically considered in relation to the distance through the meat that it is necessary for the salt to travel or, in other words, the thickness of the cut rather than its weight. The concentration of salt in the meat must be considered in relation to the weights of salt and meat; the length of time required to permit the salt to penetrate a given cut is proportional to the thickness of the meat.

On this basis the average length of cure for the hams cured with the 75-degree pickle was eight days per inch of thickness and for those with the 85-degree pickle, seven days per inch. Curing periods of this order have produced the amounts of salt in the cured hams as given in the tables. These hams appear to be on the average somewhat lower in salt content than the commercial hams studied. Further work by the authors to be reported elsewhere has indicated that curing periods of nine days per inch with an 85-degree pickle produce hams with a salinity approaching that of the commercial hams reported here.

SUMMARY

Data are presented concerning the salt content of 40 hams; of these eight were commercial hams purchased locally and the remainder were experimentally cured as follows:

(1) Twelve hams dry-cured with eight pounds of salt, three pounds of sugar, and three ounces of saltpeter applied at the rate of one ounce per pound of meat.

(2) Eleven hams brine-cured with a 75-degree sweet pickle made by dissolving the above 8-3-3 mixture in five gallons of water.

(3) Nine hams brine-cured with an 85-degree sweet pickle made by dissolving the 8-3-3 mixture in four gallons of water.

The analysis of samples taken from these hams is presented as well as the analysis of samples taken from three commercial brands of ham.

The average amount of salt present in the dry matter of the subsamples of the experimentally cured hams was practically the same for the three cures and averaged 10.29 per cent as compared with 14.59 per cent for the commercial hams, indicating that the methods used produced a milder cure under the conditions studied.

The relationship of the length of the curing period to the thickness of the ham is discussed in the case of brine-curing. Production of hams of higher salt content is possible by use of a longer curing period per inch of thickness of the meat than that used in this study.

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EFFECT OF DRYING AND STORAGE CONDITIONS ON COLOR AND SO_2 RETENTION OF DRIED APRICOTS[1]

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The SO_2 content and color of dried apricots produced in California vary considerably with the district in which the fruit is produced and the conditions under which it is dried and stored. It is a common belief that apricots dried in the cooler districts retain less SO_2 and darken more rapidly than those produced in the warmer districts. It is also a common belief that the moisture content of the fruit and the temperature at which it is stored are important factors in color and SO_2 retention.

Chace, Church, and Sorber (1933) found that apricots dried in the Merced district contained more SO_2 than apricots dried in the cooler Hollister district. They also observed that shade-drying caused variation in the SO_2 retention and quality of the dried fruit. Andersen (1929) produced dried apricots of low SO_2 content by partially drying the fruit in the shade before spreading it in the sun to complete drying. Lyon (1930) described the combination sun- and shade-drying commonly used in Australia. Jewell (1927) and Quinn (1926) stated that SO_2 absorption was more rapid by fully mature fruit than by partially mature fruit.

Cameron, *et al.* (1929), Hordh (1936a, b), Jewell (1927, 1937), Nichols and Christie (1930), and Nichols and Reed (1931) have reported a decrease in the SO_2 content of dried fruits during storage. Jewell (1937) pointed out that "moist pack" dried apricots deteriorate more rapidly during storage than "bone dry" apricots. Fisher (1937) has recently found that high moisture contents and high storage temperatures favor the darkening of dried Lovell peaches. Nichols and Reed (1931) stated that the total sulfur content of dried apricots packed in paper cartons or hermetically sealed tin containers remained fairly constant during storage, but the SO_2 gradually changed into a form that failed to inhibit darkening. Darkening occurred more rapidly when the fruit was packed in paper cartons than when packed under vacuum in tin containers. Fruit packed in an atmosphere of air in tin containers darkened more slowly than when packed under vacuum.

[1] This investigation was conducted by Professor P. F. Nichols until his death in November, 1934, then was completed by the junior authors.

The present investigation was conducted to determine the effect of locality, shade-drying, and of certain storage conditions on the retention of SO_2 and color by dried apricots.

EXPERIMENTAL PROCEDURE

Apricots were harvested and dried in the Hayward, Santa Clara (at Morgan Hill), and Aromas districts which are located in the coastal counties and the Modesto (at Ceres), Winters, and Brentwood districts located in the interior valleys. Aromas and Hayward districts have cool and often foggy nights. Modesto, Winters, and Brentwood have dry, warm, and fogless weather during the summer, while the Santa Clara district is intermediate in climate. The fresh fruit harvested in each district was divided into two lots, one of which was dried in the district where harvested and the other in one of the other districts. Fruit was exchanged, for drying, between districts having climatically different drying conditions as follows: Brentwood and Winters, Santa Clara and Aromas, and Modesto and Hayward. The drying seasons of all the districts, except those of Hayward and Modesto, overlapped sufficiently to permit simultaneous drying of the lots under comparison. The drying season of the Modesto district preceded that of the Hayward district by eight days. All fresh fruit used in the drying experiments was pitted, sulfured, and spread on the drying field within 24 hours after picking. The fruit was transferred from one district to another during the cool part of the day or at night. The drying procedure followed was similar to that commonly used in the industry, as described by Nichols (1933). The shade-drying experiments were conducted by covering trays of freshly sulfured fruit with three empty trays for a single day or until the fruit was completely dried.

Blenheim apricots were used in all tests. Partially mature fruit was separated from fully mature fruit by handpicking. The partially mature fruit was firm but well colored, whereas the fully mature fruit was soft ripe. All apricots used in the drying tests were in good condition at the time of pitting and sulfuring.

Samples of freshly sulfured apricots were obtained, when possible, immediately after the fruit was removed from the sulfur house. All samples were collected in glass jars, sealed, and stored at 0°C.(32°F.) until analyzed for SO_2 and moisture. The dried samples were collected in paper bags, taken to Berkeley, and immediately sealed in four-ounce, anchor-cap glass jars. The samples packed in jars were then stored at 0°C.(32°F.), 21.1°C.(70°F.), or 37.8°C.(100°F.) and examined periodically for changes in appearance and SO_2 content.

Storage tests were made with first-quality, freshly dried apricots containing 26.7 per cent moisture and 3,560 parts per million of SO_2. The fruit was packed in pint, slip-top, wax-paper cups and in four-ounce, anchor-cap glass jars. Several jars of the fruit were sealed under vacuum, in an atmosphere of air, of nitrogen, or of CO_2. Samples of each pack were stored at —17.8°C.(0°F.), 0°C.(32°F.), 21.1°C.(70°F.), or 37.8°C.(100°F.) and observed periodically for changes in color and SO_2 content. Fruit stored in wax-paper cartons was analyzed periodically for moisture and SO_2.

The moisture content of the samples was determined by oven-drying *in vacuo* at 70°C.(158°F.) for 12 hours. The SO_2 content was found by distillation with HCl into iodine. All SO_2 determinations were calculated and reported on the basis of fruit containing 20 per cent of moisture.

Color grades were determined by comparing the experimental samples with a series of standard color samples of dried apricots. Color comparisons were always made by two or more people familiar with the quality grades of dried apricots. Samples were graded for color numerically on the basis of merchantability of the fruit as follows: 1, 2, 3, and 4 merchantable; 5 and 6 border-line; and 7, 8, and 9 unmerchantable.

RESULTS AND DISCUSSION

Effect of District: The retention of SO_2 by apricots during drying varied with the district in which the fruit was produced or dried. Fruit dried in the warmer districts of Modesto, Brentwood, Winters, and Santa Clara retained more SO_2 and a better color than fruit dried in the cooler Hayward and Aromas districts.

When apricots were exchanged from one district to another for drying, the fruit dried in the cool Aromas and Hayward districts retained less SO_2 than fruit dried in the other districts, regardless of the origin of the fruit (Table 1). In most instances apricots dried in the cooler districts were poorer in color than those produced in the warmer districts (Table 1). During the period when drying tests were under way at Aromas and Hayward the evenings and mornings were cool and often foggy, whereas in the other districts the evenings and mornings were warm and free of fog. The drying periods ranged from five to seven days in the Aromas and Hayward districts and from three to five days in the other districts. These observations indicate that a warm drying climate in which the fruit dries rapidly favors the retention of more SO_2 and a better color than a cool, slow-drying climate. Apricots grown in the different districts undoubtedly varied in chemical composition, hence the power to retain SO_2. Apricots produced in the cooler districts and dried in the warmer

TABLE 1

Sulfur Dioxide Content of Apricots Dried in Various Districts and Stored at Various Temperatures

(Values for SO_2 given in parts per million)

Code	District in which fruit was produced	Maturity of fruit	Drying procedure	Place where dried	SO_2 in fruit when removed from sulfur house	SO_2 in dried fruit				H_2O in dried fruit
						Freshly dried fruit	Stored at 0°C. (32°F.) for 21 months	Stored at 21.1°C. (70°F.) for 15 months	Stored at 37.8°C. (100°F.) for 9 months	
B1	Brentwood	Slightly green	Standard	Brentwood	.....	1,681(4)[1]	1,590(4)	440(6)	106(8)	10.76
B2	Brentwood	Ripe	Standard	Brentwood	6,774	1,750(1)	1,660(1)	630(6)	109(8)	11.68
B3	Brentwood	Ripe	In shade	Brentwood	6,774	1,134(3)	1,100(3)	382(5)	130(6)	11.01
B4	Brentwood	Ripe	Sun 1 day, then in shade	Brentwood	6,774	1,742(4)	1,625(4)	597(4)	253(6)	11.40
B5	Brentwood	Ripe	Standard	Winters	9,940	1,877(1)	1,632(1)	436(7)	.....(8)	12.78
B6	Brentwood	Slightly green	Standard	Winters	.....	2,089(4)	2,100(4)	653(7)	128(9)	11.77
W1	Winters	Slightly green	Standard	Winters	.....	1,788(1)	1,630(1)	305(8)	109(8)	13.25
W2	Winters	Ripe	Standard	Winters	11,700	2,650(3)	2,600(3)	785(6)	104(9)	13.82
W3	Winters	Ripe	In shade	Winters	.....	1,650(3)	1,472(3)	370(5)	85(9)	12.54
W4	Winters	Ripe	Sun 1 day, then in shade	Winters	.....	2,420(1)	2,260(1)	642(6)	116(9)	15.30
W5	Winters	Slightly green	Standard	Brentwood	10,490	2,128(3)	2,125(3)	475(4)	119(8)	13.20
W6	Winters	Ripe	Standard	Brentwood	10,490	2,120(3)	1,985(3)	730(6)	135(7)	11.68
C1	Modesto	Slightly green	Standard	Ceres	7,340	1,515(2)	1,390(2)	620(4)	152(4)	11.40
C2	Modesto	Ripe	Standard	Ceres	7,340	1,400(1)	1,127(2)	460(4)	319(6)	10.28
C3	Modesto	Ripe	In shade	Ceres	.....	740(3)	655(3)	322(5)	85(8)	12.84
C4	Modesto	Ripe	Sun 1 day, then in shade	Ceres	.....	2,220(2)	2,100(2)	815(4)	467(5)	20.27
C5	Modesto	Slightly green	Standard	Hayward	9,410	890(3)	786(3)	265(5)	110(8)	13.19
C6	Modesto	Ripe	Standard	Hayward	9,100	1,080(3)	1,022(3)	328(5)	.....(7)	12.39
H1	Hayward	Slightly green	Standard	Hayward	.....	514(5)	464(5)	51(7)	117(8)	18.75
H2	Hayward	Ripe	Standard	Hayward	.....	564(4)	484(5)	83(8)	119(8)	16.74
H3	Hayward	Ripe	In shade	Hayward	.....	394(5)	370(5)	42(8)	120(8)	16.63
H4	Hayward	Ripe	Sun 1 day, then in shade	Hayward	.....	522(4)	463(5)	43(8)	48(8)	14.43
H5	Hayward	Slightly green	Standard	Ceres	.....	867(1)	800(6)	95(8)	119(8)	10.74
H6	Hayward	Ripe	Standard	Ceres	.....	1,100(1)	1,025(4)	137(7)	127(8)	10.76
M1	Santa Clara	Ripe	Standard	Morgan Hill	11,370	1,350(4)	1,130(4)	500(5)	128(6)	13.86
M2	Santa Clara	Ripe	In shade	Morgan Hill	11,600	1,090(4)	953(4)	159(6)	165(7)	14.94
M3	Santa Clara	Ripe	Sun 1 day, then in shade	Morgan Hill	11,600	1,370(4)	1,253(5)	380(5)	165(7)	14.26
M4	Santa Clara	Ripe	Standard	Aromas	6,320	936(5)	710(5)	197(6)	112(7)	15.55
A1	Aromas	Ripe	Standard	Aromas	3,460	415(5)	340(5)	30(7)	132(8)	17.71
A2	Aromas	Ripe	In shade	Aromas	3,440	202(5)	180(5)	13(8)	40(8)	19.11
A3	Aromas	Ripe	Sun 1 day, then in shade	Aromas	4,540	365(4)	344(4)	23(7)	173(8)	17.19
A4	Aromas	Ripe	Standard	Morgan Hill	6,790	910(1)	665(4)	197(7)	178(8)	13.28

[1] Numbers in parentheses refer to color grade.

districts retained less SO_2 than fruit harvested and dried in the warmer districts. On the other hand, apricots produced in the warmer districts and dried in the cooler districts retained more SO_2 than apricots grown and dried in the cooler districts. The color grade of the freshly dried fruit varied with SO_2 retention during drying (Table 1).

Drying tests made with partially and fully mature fruit did not yield consistent results with relation of maturity to SO_2 and color retention. However, data obtained from a more detailed investiga-

TABLE 2

Effect of Storage Temperature on Changes in SO_2, Moisture, and Color of Dried Apricots Stored in Wax-Paper Cups

(Values for SO_2 given in parts per million)

Storage period	Storage temperatures							
	—17.8°C.(0°F.)		0°C.(32°F.)		21.1°C.(70°F.)		37.8°C.(100°F.)	
	SO_2	Moisture	SO_2	Moisture	SO_2	Moisture	SO_2	Moisture
days		*pct.*		*pct.*		*pct.*		*pct.*
0	3,540	26.7(1)[1]	3,560	26.7(1)	3,560	26.7(1)	3,560	26.7 (2)
7	.....	.....(2)	.....	....	.....	....	2,000	17.0 (2)
14	.....	.....(2)	.....	....	.....	....	2,200	11.4 (2)
21	.....	.....(2)	.....	....	.....	....	1,905	9.85(2)
30	3,090	24.3(2)	3,010	23.5(2)	2,180	22.3(2)	2,118	8.3 (2)
60	3,088	23.4(2)	2,640	21.6(2)	1,610	14.3(2)	1,900	7.9 (3)
90	2,960	24.3(2)	2,700	21.8(2)	1,310	15.4(2)	1,530	7.9 (4)
120	2,940	23.8(2)	2,565	21.4(2)	1,088	13.4(2)	1,448	7.4 (4)
150	.....	.....(2)	.....	.....(2)	1,050	11.4(2)	1,190	6.3 (5)
390	.....	.....(2)	.....	.....(2)	480	7.5(7)	723	5.0 (8)

[1] Numbers in parentheses refer to color grade.

tion now in progress indicates that SO_2 retention varies with the maturity, variety, and chemical composition of the fruit to be dried.

Effect of Shade-Drying: Complete shade-drying increased the drying time and reduced the SO_2 retention in all districts. The appearance of shade-dried fruit was fair in the warmer districts and poor in the cooler districts. Fruit exposed to the sun for one day and then dried in the shade retained SO_2 and color better than fruit completely dried in the shade. Exposure of the sulfured fruit to the sun for one day was sufficient in the warmer districts to prevent the loss of SO_2 that occurred when apricots were completely dried in the shade. This practice however, is not recommended as a commercial procedure because of variations with climatic conditions.

Effect of Storage Conditions: The loss of SO_2 during storage increased with increase in temperature and storage period. Color deterioration was most rapid at the higher storage temperature (Tables

[1] Numbers in parentheses refer to color grade.

TABLE 3

Effect of Storage Conditions on Changes in SO_2 Content and Color of Dried Apricots

(Values for SO_2 given in parts per million)

Storage period	In atmosphere of nitrogen in glass jars				In vacuum in glass jars				In atmosphere of air in glass jars				In atmosphere of CO_2 in glass jars			
	Storage temperatures				Storage temperatures				Storage temperatures				Storage temperatures			
	—17.8°C. (0°F.)	0°C. (32°F.)	21.1°C. (70°F.)	37.8°C. (100°F.)	—17.8°C. (0°F.)	0°C. (32°F.)	21.1°C. (70°F.)	37.8°C. (100°F.)	—17.8°C. (0°F.)	0°C. (32°F.)	21.1°C. (70°F.)	37.8°C. (100°F.)	—17.8°C. (0°F.)	0°C. (32°F.)	21.1°C. (70°F.)	37.8°C. (100°F.)
	SO_2	SO_2	SO_2	SO_2	SO_2	SO_2	SO_2	SO_2	SO_2	SO_2	SO_2	SO_2	SO_2	SO_2	SO_2	SO_2
days																
0	3,560(1)[1]	3,560(1)	3,560(1)	3,560(1)	3,560(1)	3,560(1)	3,560(1)	3,560(1)	3,560(1)	3,560(1)	3,560(1)	3,560(1)	3,560(1)	3,560(1)	3,560(1)	3,560(1)
7	.....(1)	.....(1)	.....(1)	3,103(1)	.....(1)	.....(1)	.....(1)	3,390(1)	.....(1)	.....(1)	.....(1)	2,930(1)	.....(1)	.....(1)	.....(1)	2,975(1)
14	.....(1)	.....(1)	.....(1)	3,080(1)	.....(1)	.....(1)	.....(1)	3,216(1)	.....(1)	.....(1)	.....(1)	2,860(1)	.....(1)	.....(1)	.....(1)	2,935(1)
21	.....(1)	.....(1)	.....(1)	2,802(1)	.....(1)	.....(1)	.....(1)	3,252(1)	.....(1)	.....(1)	.....(1)	2,740(1)	.....(1)	.....(1)	.....(1)	2,830(1)
30	3,570(1)	2,970(1)	3,020(1)	2,975(1)	3,430(1)	3,240(1)	3,148(1)	3,204(1)	3,240(1)	3,322(1)	3,040(1)	2,800(1)	3,362(1)	3,220(1)	3,105(1)	2,760(1)
60	3,240(1)	3,550(1)	3,110(1)	2,548(1)	3,258(1)	3,215(1)	2,935(1)	2,560(1)	3,250(1)	3,321(1)	2,858(1)	2,518(1)	3,260(1)	3,200(1)	2,890(1)	1,988(1)
90	3,240(1)	3,500(1)	2,720(1)	2,040(1)	3,180(1)	3,242(1)	2,562(1)	2,080(1)	3,235(1)	3,038(1)	2,900(1)	2,078(1)	3,220(1)	3,045(1)	2,670(1)	1,892(1)
120	3,280(1)	3,080(1)	2,780(1)	1,880(2)	3,302(1)	3,198(1)	2,778(1)	1,670(3)	3,198(1)	2,950(1)	2,621(1)	1,860(2)	2,947(1)	2,980(1)	2,730(1)	1,751(2)
150	.....(1)	.....(1)	.....(1)	1,100(3)	.....(1)	.....(1)	.....(1)	1,595(3)	.....(1)	.....(1)	.....(1)	1,450(3)	.....(1)	.....(1)	.....(1)	1,480(3)
390	.....(1)	.....(1)	.....(2)	642(8)	.....(1)	.....(1)	.....(1)	920(8)	.....(1)	.....(1)	.....(1)	1,102(8)	.....(1)	.....(1)	.....(2)	1,172(8)
420	3,027(1)	3,060(1)	1,780(3)	219(9)	....	....	....	307(9)	2,988(1)	2,922(1)	1,430(4)	327(9)	3,333(1)	3,158(1)	1,360(4)	344(9)
560	....	....	1,120(5)	....	....	....	1,169(6)	....	....	....	1,162(6)	....	....	....	1,136(6)	....

[1] Numbers in parentheses refer to color grade.

1 and 2). Dried fruit from the Aromas and Hayward districts deteriorated in color more rapidly than fruit dried in the other districts.

Dried apricots stored in paper cups decreased in SO_2 and color grade more rapidly than fruit sealed in four-ounce jars (Tables 2 and 3). Apricots stored in paper cups at 21.1°C.(70°F.) and 37.8°C. (100°F.) decreased in moisture content rapidly, whereas those stored in jars retained 26.7 per cent of moisture throughout the experiment. Fruit stored in paper cups at 21.1°C. decreased in SO_2 content more rapidly but in color grade more slowly than when stored in paper cups at 37.8°C. Fruit stored in jars, on the other hand, decreased in SO_2 content more rapidly when stored at 37.8°C. than when stored at 21.1°C. These variations in SO_2 content can be attributed to the rapid loss of moisture in paper-cup samples stored at 37.8°C. as compared with paper-cup samples stored at 21.1°C. or the jar samples which did not change in moisture content. The rapid loss of moisture by the paper-cup samples stored at 37.8°C. apparently retarded the loss of SO_2 but did not prevent color deterioration.

Samples packed in glass jars under various atmospheric conditions all decreased in SO_2 content and color grade similarly when stored under similar conditions. It is apparent that an increased storage temperature caused a more rapid decrease in the SO_2 content of the fruit even though the jars were sealed throughout the storage periods (Table 3). In the tests conducted, temperature was a more important factor than the atmosphere in which the fruit was stored. It is possible, however, that sufficient air remained occluded in the fruit to cause oxidative changes in spite of the non-oxidizing nature of two of the gases and the vacuum treatments. Another possibility is that certain constituents of the fruit acted as oxidizing agents and in turn were reduced by the SO_2. The glass containers were sealed sufficiently tight to prevent the loss of SO_2, in the form of gas, during storage.

The color changes of the samples stored under various atmospheres were similar in all instances (Table 3). Time and temperature of storage were more important factors in color deterioration than the type of atmosphere surrounding the fruit.

CONCLUSIONS

1. Color and SO_2 retention by dried apricots vary with the climatic conditions at the time the fruit is dried. Apricots dried in warm and relatively dry districts retain more SO_2 and a better color than those dried in cool and foggy districts.

2. Complete drying in the shade increases the drying time and reduces SO_2 retention by the fruit.

3. The decrease in SO_2 content during storage of dried apricots varies with the storage temperature, length of storage period, and the moisture content of the fruit. Atmospheres of air, nitrogen, or CO_2 or a vacuum do not prevent the decrease in SO_2 content or color deterioration of the fruit during storage.

4. The color grade of dried apricots varies with the drying conditions under which the fruit is produced and the temperature at which it is stored.

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OXALATES IN PINEAPPLES[1]

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From a review of the literature it appears that incorrect interpretations of analytical results have caused much confusion regarding the oxalate content of pineapples *(Ananas comosus* [L.] *Merr.)*. An anonymous report (1936) and an editorial (1934) stating that pineapple contained "strikingly high" amounts of oxalic acid led to a search for data which might justify such statements.

Kohman (1934) published analyses of pineapple fruit and juice, giving oxalic acid concentrations ranging from .007 to .03 per cent of their fresh weight. He also referred to published implications not supported by quantitative data that pineapples contained high amounts of oxalates, but definitely stated "pineapples contain no more oxalate than many other common fruits and rank low in calcium oxalate as compared with a number of other foods."

The subsequent editorial (1934), although quoting the correct oxalate values from Kohman's paper, made this declaration, "Almost every fruit and vegetable contains oxalic acid; pineapple and leafy vegetables, notably spinach, are strikingly high in this respect (from 0.01 to 0.03 and from 0.29 to 0.69 per cent respectively)." Neither these data nor those from many other different foods furnish any basis for the allegation that oxalates are present in "high" concentrations in pineapples.

In view of the questionable statements quoted a repetition and publication of analyses for oxalates in pineapple seemed desirable. The variety employed was Cayenne, the one most widely grown in the Hawaiian Islands. The results are herewith reported, together with a description and critical evaluation of the technique employed.

HISTOLOGICAL OBSERVATIONS

The presence in fresh fruit of crystals resembling raphides of calcium oxalate has been mentioned by Miller (1928). These crystals are insoluble in glacial acetic acid but are soluble in tenth-normal hydrochloric. Upon the addition of concentrated sulphuric acid characteristic crystals of calcium sulphate slowly form. That the

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raphides are calcium oxalate was further confirmed by analyses for oxalates in a heterogeneous precipitate including many of these crystals obtained by centrifuging the juice from several thoroughly ground pineapples. Of this precipitate when air dry, 29 per cent could be accounted for as anhydrous calcium oxalate by the methods outlined below. Attempts at quantitative determination of total oxalates in aliquots of tissue were then made.

ANALYTICAL TECHNIQUE

Preparation of Tissue for Analysis: In preparation for total oxalate analyses the tissue was acidified with 1.6 milliequivalents of hydrochloric acid per gram of tissue, and the soft fruit pulp was thoroughly ground in a mortar. The resulting suspension had a pH of less than .4. Subsequent microscopic examination of pulp and precipitate failed to show the presence of any raphides. That insoluble calcium oxalate was converted into soluble oxalic acid under these conditions was further established by the addition of prepared calcium oxalate to fruit pulp thus acidified. Concentrations of calcium oxalate equivalent to .078 per cent of the tissue as oxalic acid were recovered as such when added to pineapple juice containing amounts of oxalic acid too small to be determined by the procedures herewith reported.

The acidified pulp was diluted to an appropriate volume (50 gm. of tissue to 100 c.c.), centrifuged, and filtered. The filtrate was sometimes heated in order to precipitate more colloidal material and subsequently filtered, but this was generally not necessary.

In order to remove oxalic acid from the presence of organic substances possibly interfering with precipitation of calcium oxalate, preparation of extracts by the method of Pucher, Vickery, and Wakeman (1934) was also attempted. Calculations, based on the size of aliquots and on the solubility of calcium oxalate as reported by Schlesinger and Wallen (1929), indicated that .01 per cent oxalic acid should be detected in extracts prepared in this manner. No detectable precipitate of calcium oxalate, however, could be obtained.

Chemical Methods: Aliquots of 25 c.c. of the clear filtrate or of the alkaline aqueous solution prepared by the ether extraction technique of Pucher, Vickery, and Wakeman (1934) were neutralized to methyl red. To prevent the precipitation of calcium citrate 3 c.c. of glacial acetic acid were added to each aliquot, giving a pH of about 3. Calcium oxalate was then precipitated by the addition of 5 c.c. of a 10-per cent calcium chloride solution. The precipitate was allowed to stand overnight and was then filtered off on asbestos in a Gooch crucible, being washed with a .3-normal solution of ammonium

hydroxide. The calcium oxalate was then dissolved in 25 c.c. of boiling 10-per cent sulphuric acid to which the filter pad was also added; and the oxalic acid was determined by titration while hot, with .02-normal potassium permanganate.

RECOVERY OF OXALIC ACID

It is known from the work of Shohl (1922) that the solubility of calcium oxalate increases below pH 4, but the presence of high amounts of citric acid made necessary the precipitation at pH 3. Recoveries of oxalic acid from an aqueous solution when the above technique was used are shown (Table 1). Losses of oxalic acid, except when the amounts added were very low, do not indicate that the solubility of calcium oxalate was appreciably increased over that

TABLE 1

Recovery of Oxalic Acid Added to Water [1]

Added	Recovered	Lost	Recovery
mg.	*mg.*	*mg.*	*pct.*
0.6	0.09	.51	15
1.2	1.04	.16	87
1.2	1.08	.12	90
1.2	1.08	.12	90
3.0	2.70	.30	90

[1] Precipitation of calcium oxalate below pH 3 in 50-c.c. volume.

in water alone. This value, 96.9×10^{-6} equivalents per liter at 25°C. (77°F.), as given by Schlesinger and Wallen (1929), is equivalent to .218 mg. of oxalic acid in 50 c.c. On the basis of the aliquots employed this solubility would correspond to .0017 per cent of the fresh weight of the tissue. Acetic acid apparently interfered with the formation of a crystalline precipitate of calcium oxalate only when oxalic acid was present in extremely low concentrations. When acetic acid was omitted, recovery was 82 per cent from the solution including .6 mgm. oxalic acid, but recoveries from the more concentrated oxalic acid solutions were not significantly higher than when acetic acid was present.

Recoveries of low concentrations of oxalic acid added to pineapple juice closely corresponding to those in Table 1 are given (Table 2). Determinations on the juice alone indicated the presence of extremely small amounts of oxalic acid. It is apparent that the presence of .6 mg. or slightly less of oxalic acid per aliquot, equivalent to .0048 per cent of the fresh tissue, could be qualitatively established. The method gave quantitative recoveries only when concentrations equivalent to .024 per cent or more of the fresh tissue were employed.

It should be added that traces of organic matter from the juice were precipitated along with the calcium oxalate. Oxidation of the contaminant by permanganate would result in slightly high values for oxalic acid in the juice alone.

In only one case among many pineapple fruits analyzed by the above method has a value exceeding or even approaching .005 per cent oxalic acid been obtained. The one exception was a determination of .008 per cent.

The exact determination of the oxalic acid present must await the development of a reliable micro-method accurate within the range

TABLE 2

Recovery of Oxalic Acid Added to Pineapple Juice [1]

Oxalic acid in aliquot						Oxalic acid in fresh tissue corresponding to amount added to aliquot
In juice [2]	Added	Found	Lost	Recovered	Recovery	
mg.	*mg.*	*mg.*	*mg.*	*mg.*	*pct.*	*pct.*
.14	0.6	0.19	.55	0.05	7	.0048
.14	0.6	0.29	.45	0.15	25	.0048
.14	1.2	1.13	.21	0.99	82	.0096
.14	1.2	1.18	.16	1.04	87	.0096
.14	1.8	1.72	.22	1.58	88	.0144
.14	1.8	1.81	.13	1.67	93	.0144
.00	3.0	2.97	.03	2.97	99	.024
.00	3.0	3.15	—.15	3.15	105	.024
.00	6.0	6.04	—.04	6.04	101	.048
.00	6.0	6.20	—.20	6.20	103	.048
.31	7.2	7.56	—.05	7.25	101	.058

[1] Precipitation of calcium oxalate at pH 3 in 50-c.c. volume. [2] Aliquot equivalent to 12.5 grams of original fresh juice or fruit tissue.

of concentrations indicated by the above experiments which show that ripe Cayenne pineapples certainly contain less than .01 per cent and probably less than .005 per cent oxalic acid as oxalates in any form. It is apparent that the concentration is so low that it has no physiological importance in human nutrition, as noted by Kohman (1934).

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FLAVOR CONTRIBUTORS OF SWISS-TYPE CHEESE[1]

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Different types of cheese have flavors that are more or less characteristic. With each type the general flavor is due to a combination of flavors contributed by certain chemical compounds produced in the cheese. A given lot of milk can be made into any variety of cheese desired so that the changes in the milk constituents during the manufacturing and ripening processes are more or less typical of each variety. Although the enzymes normally present in the milk and in the rennet extract sometimes play a part in bringing about these changes, microörganisms are of special significance and certain cheeses have organisms that are more or less characteristic of them.

Swiss-type cheese of good quality has a sweet flavor, as described by Doane (1932) and Sherman (1921, 1923). Freudenreich and Orla-Jensen (1906) attributed this to amino acids, since these are formed in comparatively large amounts during the ripening and some of them are sweet. Amino acids are also liberated in relatively large amounts in various cheeses that are not characterized by a sweet flavor; however, Swiss-type cheese may present a set of conditions under which certain of these acids tend to accumulate.

At the Iowa Agricultural Experiment Station the ripening of a Swiss-type cheese made from pasteurized milk is being investigated. With this cheese the addition of certain strains of propionic acid organisms (genus *Propionibacterium*) to the pasteurized milk is very beneficial from the standpoint of development of the desired sweet flavor so that the situation is much the same as with the orthodox Swiss cheese. The cheese made without addition of propionic acid organisms to the milk often lacks the sweet flavor; when it shows the desired flavor, propionic acid organisms can be found in considerable numbers.

The correlation between sweet flavor of the cheese and presence of large numbers of propionic acid organisms suggests that certain products formed by the organisms are of special significance as flavor contributors. The general information available on these organisms indicates that one of the characteristic things about them is the production of propionic and acetic acids from lactic acid or lac-

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tates, the general reaction being as follows: 3 CH_3-CHOH-COOH $\longrightarrow$ 2 CH_3-CH_2-COOH + CH_3-COOH + CO_2 + H_2O.

Propionic and acetic acids as such do not have sweet flavors, so that various combinations of them should be considered. The acids produced in Swiss-type cheese would be expected to combine with various materials present, particularly with calcium, potassium, and sodium. When dry calcium propionate is placed on the tongue it suggests first a sweet flavor and then a bitter flavor. Dry potassium and sodium propionates also give an impression of sweetness. The acetates of calcium, potassium, and sodium have very little sweet flavor.

TABLE 1

Comparative Amounts of Volatile Acids in Swiss-Type Cheese of Different Qualities

Source of cheese	Flavor	N/20 acid in first 1,000 ml. distillate from 400 grams of cheese
		ml.
Experimental[1]; control	No sweet flavor.	30.4
Experimental[1]; milk inoculated with propionic acid organism	Considerable sweet flavor.	381.2
Experimental[1]; control	Very little sweet flavor.	88.4
Experimental[1]; milk inoculated with propionic acid organism	Considerable sweet flavor.	398.0
Market; high-quality domestic	Considerable sweet flavor.	238.8
Market; high-quality imported	Considerable sweet flavor.	175.0
Market; low-quality domestic	Very little sweet flavor.	65.0
Market; low-quality domestic	Very little sweet flavor.	72.5
Market; fair-quality domestic	Considerable sweet flavor.	221.0

[1] Made from pasteurized milk.

EXPERIMENTAL PROCEDURE

Because of the difficulty of determining calcium, potassium, and sodium propionates in cheese directly, the volatile acidities of Swiss-type cheese of different qualities were estimated on a comparative basis by distilling cheese after adding a small amount of sulfuric acid to aid in freeing the volatile acids. The cheese was shredded, 400 grams were weighed into a distillation flask, 20 ml. N/1 sulfuric

acid was added, and steam was turned into the flask after placing it on a water bath and connecting it with a condenser. The first 1,000 ml. of distillate was titrated to the phenolphthalein end point with N/20 sodium hydroxide.

In general, the data show a much higher content of volatile acids in cheese having considerable sweet flavor than in cheese having little or no sweet flavor (Table 1). This suggests that combinations of the volatile acids are related to the sweet flavor and, presumably, propionic acid is of more importance in this connection than acetic acid.

TABLE 2

Effect of Added Calcium and Sodium Propionates on Flavor of Process Swiss-Type Cheese

Propionate added	Flavor
None	No sweet flavor.
0.25% calcium propionate	Very little sweet flavor.
0.5 % calcium propionate	Definite sweet flavor.
1.0 % calcium propionate	Distinct sweet flavor.
2.0 % calcium propionate	Considerable sweet flavor.
0.25% sodium propionate	No sweet flavor.
0.5 % sodium propionate	Very little sweet flavor.
1.0 % sodium propionate	Very little sweet flavor.
2.0 % sodium propionate	Distinct sweet flavor.
None	No sweet flavor.
0.75% calcium propionate	Definite sweet flavor.
1.0 % calcium propionate	Distinct sweet flavor.
1.5 % calcium propionate	Considerable sweet flavor.
0.75% sodium propionate	Very little sweet flavor.
1.0 % sodium propionate	Very little sweet flavor.
1.5 % sodium propionate	Definite sweet flavor.

The effects of calcium and sodium propionates on the flavor of process Swiss-type cheese were investigated by adding these compounds to the cheese during the processing. The cheese selected for the trials was very lacking in flavor so that any influence of the propionates would be more conspicuous. Appropriate controls, without added propionate, were also prepared. The addition of a propionate just before the cheese was drawn from the kettle was much more satisfactory from the standpoint of maintaining a homogeneous condition in the cheese than adding it early in the processing.

The addition of the propionates strikingly influenced the flavor of the cheese (Table 2). Calcium propionate had a more pronounced effect than sodium propionate, although the effect of the latter was

very definite. With either salt an increase in the percentage added increased the sweet flavor in a more or less regular manner. In one instance the addition of one per cent alanine, which was decidedly sweet, did not change the flavor of the cheese significantly.

The larger amounts of propionate not only influenced the flavor of the cheese but also resulted in a body which was more like the body of natural Swiss-type cheese than was the body of the control cheese. The color was also lighter than that of the cheese without the added propionate.

DISCUSSION

The relatively high volatile acidity of Swiss-type cheese having a conspicuous sweet flavor, compared with the volatile acidity of cheese lacking a definite sweet flavor, indicates the importance of the volatile acids or their salts from the standpoint of desired flavor development. The effects of adding propionates to process Swiss-type cheese also suggest the influence of these salts on the flavor, although added propionates presumably would not act exactly as propionates produced in the cheese. During the ripening of Swiss-type cheese propionic acid is produced through bacterial action and, because of the opportunities for reactions, various propionates are formed. The reaction with calcium is probably the most important from the quantitative standpoint. Although acetic acid is also produced during the ripening of Swiss-type cheese, the acetates would not be expected to contribute significantly to the characteristic flavor.

The change in the texture and color of process Swiss-type cheese as a result of the addition of propionates during the processing also suggests that the formation of propionates is an important part of the ripening of the cheese.

SUMMARY

Swiss-type cheese with considerable of the characteristic sweet flavor yielded a much higher volatile acidity on steam distillation than cheese lacking in flavor. The addition of calcium or sodium propionate to process Swiss-type cheese lacking in flavor gave the product a sweet flavor; also, the texture was more like natural Swiss cheese and the color was lighter. These results suggest that propionates, of which calcium propionate is presumably the most significant, are important flavor contributors of Swiss-type cheese.

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VITAMIN C CONTENT OF SOME TEXAS FRUITS AND VEGETABLES

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Vitamin C has been isolated and analyzed and is now usually called ascorbic acid. Up to 1932 the only known method of determining vitamin C in a material was by biological assay, by which the minimum dosage was found which when administered daily would prevent the animal treated from developing scurvy. Tillmans, Hirsch, and Jackisch (1932) showed that a standardized aqueous solution of the dye 2,6-dichlorophenolindophenol is quantitatively reduced to a colorless product by ascorbic acid. Birch, Harris, and Ray (1933), Bessey and King (1933), Mack and Tressler (1937), and others have published modifications of Tillmans' method involving microamounts and titration in acid medium.

EXPERIMENTAL PROCEDURE

The method of Bessey and King and a modification of the extracting solution suggested by Mack and Tressler (1937) were used in the work reported here. The method, briefly, is to triturate a 2.5-gram sample with acid-washed sand and 15 ml. of a two-per cent HPO_3-5% H_2SO_4 solution, filter the liquid through a Büchner funnel, and re-extract the residue and paper with 10 or 12 ml. of the mixed acid solution. The filtrate is collected in a 100-ml. volumetric flask, and made up to volume with eight-per cent acetic acid. After shaking, 25-ml. aliquots are titrated with the dye solution from a microburette. A blank containing only the acids is run. The dye solution containing .01 gram of dye in 50 ml. of water is standardized by titration with a freshly prepared solution of .1 gram of ascorbic acid in 100 ml. of water, which in turn has been previously standardized with .01 N iodine solution. The iodine solution is standardized with arsenious oxide from the U. S. Bureau of Standards.

The amounts of ascorbic acid in 217 various fruits and vegetables grown in Texas in 1937 are recorded (Table 1). Mustard, peppers, turnip greens, and small-headed cabbage are especially high in vitamin C, containing over 100 milligrams per 100 grams. Turnips, per-

TABLE 1

Ascorbic Acid in Texas Fruits and Vegetables, and Amount of Each Food Necessary to Furnish 20 Milligrams of Ascorbic Acid

Kind of food	Individual analyses	Average	Food required to furnish 20 mg. ascorbic acid
	mg. per 100 gm.	*mg. per 100 gm.*	*gm.*
Cabbage, small-headed	130, 132, 131, 158, 100	130.0	15
Cantaloupes			
Hale's Best	31.8, 35.5, 32.5	33.3	60
Hearts of Gold	26.5, 22, 17.7, 17.7, 32.1, 30	24.3	82
Honey Ball	30.7	30.7	65
Honey Dew	7.3	7.3	274
Netted Gem	37.1	37.1	54
Rocky Ford	31, 19.3, 22.6, 24.8, 26.4, 34.5	26.4	76
Carrots	8.4	8.4	238
Eggplant	2.5, 1.8, 2.9, 1.9, 1.5	2.1	950
Figs	2.5, 3.5, 1, 2.9, 0.7, 1.6, 1.5	1.8	1,111
Grapes			
Green	1.2, 2.6	1.9	1,053
Muscadine (pulp)	5.5, 1.9	3.7	540
Scuppernong	7.2, 7.2, 7.2	7.2	278
Small pink	2.3	2.3	871
Grapefruit			
Conners' Prolific	38.9, 34.6	36.8	54
Duncan	36.6, 37.4	37.0	54
Foster Pink	41.7, 41.5, 49.1	44.1	45
Marsh Seedless	23.6, 24.3, 41.7, 35.6	31.3	64
McCarty	31.5, 31	31.3	64
Thompson or Pink Marsh	30.4, 33.4, 37.4, 37.4	34.7	58
Lemons (pulp)			
Eureka	44.6, 44.6	44.6	45
Green rind	24.8, 24, 42.2, 29.6	30.1	66
Large, green rind	18.2, 13, 16.6	15.9	126
Large, yellow	9.9, 10.1, 14.2	11.4	175
Meyer Seedless	9.9, 11.8, 18.7, 17.5	14.5	138
Round yellow, thin rind	14.8, 22, 27.6	21.5	93
Yellow, medium size	26.5, 21.4, 30.7	26.2	76
Limes (pulp)			
Mexican—(gath. Aug. 1)	16	16.0	125
Mexican—(gath. Oct. 15)	19.8, 20.5, 17.5, 26.9, 22.5	21.5	93
Rangpur	7.4, 8.1, 12.9, 9.4	9.5	212
Muskmelons, big	7.3, 11.2, 18	12.2	164
Mustard	158, 171.1	165.0	13
Onions, Bermuda	6.3, 4.5, 9.9, 9.5, 4.9, 8.7	7.3	274
Oranges			
Hamlin	36.8, 44.3, 46.7, 45.9	43.4	46
Temple	48.8, 37.2, 32.1, 36.2	38.6	52
Texas Navel (seedless)	43.5, 48.6	46.1	43
Valencia	37.8, 37.7, 35.7, 36.9	37.0	54
Washington Navel (seedless)	30.3, 29.2	29.2	67
Calamondin	31.5, 36, 37.8	35.1	57
Peaches			
Elberta	3.5, 1.7, 1.5	2.2	909
Fall Yellow Clearseed	14.8, 10.9	12.9	155
Green Cling	15	15.0	133
White Clearseed	7, 7.8	7.4	270
Yellow Cling	13.3	13.3	150

TABLE 1 *(Concluded)*

Ascorbic Acid in Texas Fruits and Vegetables, and Amount of Each Food Necessary to Furnish 20 Milligrams of Ascorbic Acid

Kind of food	Individual analyses	Average	Food required to furnish 20 mg. ascorbic acid
	mg. per 100 gm.	*mg. per 100 gm.*	*gm.*
Pears			
Garber	1.8, 1.8, 3.2	2.3	871
Kieffer	3.1, 2.1, 2.3	2.5	800
Peppers			
Florida Gem, green ripe	128.5	128.5	16
Florida Gem, red ripe	195.6	195.6	10
Hungarian Dox, green ripe	140.1	140.1	14
Hungarian Dox, red ripe	252.8	252.8	8
Neapolitan, green ripe	131.4, 197.3	164.4	12
Neapolitan, red ripe	177.5, 279	228.3	9
Ruby King, red ripe	281	281.0	7
Small, blunt, green pod	104.4	104.4	19
Small, slender, green pod	140	140.0	14
Persimmons (pulp)	32.4, 53, 44	43.1	46
Plums			
Large, yellow	2.6, 2.7, 2, 2.2	2.4	833
Small, pink	3.5, 7.8, 10.7, 4.6	6.7	299
Wild, red	8.4	8.4	238
Pomegranates (juice)	6	6.0	333
Potatoes			
Sweet, Porto Rico (without peeling)	15.4, 19.7, 25.9	20.3	98
Shallots			
Stem (green)	26.9, 17.6	22.3	90
Bulb (white)	7.6, 7.8	7.7	260
Tangerines			
Tangelo, Pumelo Cross	30.3, 35.5	32.9	61
Tomatoes	19.9, 10.6, 12.7, 18.2, 28.2	17.9	112
Turnips (root)	53.2, 40.2, 54.4, 40.6	47.1	43
Turnip greens	129, 152.5, 157.4, 164.7, 209.8	162.7	12
Watermelons			
Cletex	8.6, 9.5	9.1	222
Cuban Queen	5.4, 5.1, 6.6, 7.4, 7.8	6.5	308
Dixie Queen (yellow meat)	4.3, 4.5	4.4	455
Halbert Honey	4.8, 7	5.9	339
Irish Gray (red meat)	8, 7	7.5	267
Irish Gray (yellow meat)	6.4	6.4	313
Parker	3.7, 4.4, 6.1, 5.9, 6.9, 6.5, 5.3	5.5	364
Stone Mountain	2.7, 3.1, 2.3, 2.7	2.7	741
Tom Watson	3.4, 3.4, 2.2, 5, 7.1, 7.5, 4.4	4.7	426

simmons, oranges, grapefruit, tangerines, cantaloupes, and lemons come next in quantitative occurrence of vitamin C, containing about 25 to 50 milligrams per 100 grams. Sweet potatoes, limes, shallot stems, tomatoes, and fall cling peaches are useful sources of vitamin C. The sweet potato, owing to its extensive cultivation and use and the large quantity eaten, is an important source of vitamin C in the popular diet. Ascorbic acid is a possible by-product in the large-scale production of sweet-potato starch.

Some striking variations are indicated in the ascorbic acid content of different varieties of the same fruit or vegetable although larger numbers of some of the varieties are needed in order to make positive statements. Honey dew melon may contain much less ascorbic acid than the other varieties of cantaloupes. Foster Pink is highest in ascorbic acid of the grapefruits, Eureka highest of the lemons, and Ruby King may be highest of the peppers. Mexican limes contain appreciably more vitamin C than Rangpur; Texas Navel (seedless) oranges may contain more than Washington Navel (seedless); and Cletex and Irish Gray watermelons may contain much more than Stone Mountain.

These results have been compared with the ascorbic acid contents of various foods compiled by Daniel and Munsell (1937). Many of the results come within the range of those reported, but there are some notable exceptions. Cantaloupes, mustard, peaches, and persimmons contain more ascorbic acid than the samples previously reported.

According to Schultzer (1937) early experience in the British navy showed that one ounce of lemon juice, corresponding to 15 mg. ascorbic acid, was the daily dose required to prevent adults from contracting scurvy. If the ascorbic acid (or vitamin C) requirement is estimated at 20 mg. daily per person, as has been done by Gothlin and other workers quoted by Schultzer (1937), it is seen that this requirement can easily be satisfied by the use of the proper vegetables or fruits. The daily intake of each food required to supply 20 mg. of vitamin C, if that food were the only source of vitamin C taken, is listed (Table 1). Sixty grams of cantaloupe, grapefruit, or orange alone will suffice to supply the daily ascorbic acid requirement.

SUMMARY

The quantities of vitamin C (ascorbic acid) were determined by titration with 2,6-dichlorophenolindophenol in 217 samples of Texas foods, and the results are expressed as milligrams per 100 grams of food. Cabbage (130), mustard (165), peppers (104 to 281), and turnip greens (162), contained the highest quantities of ascorbic acid. Next came cantaloupes (7.3 to 37), grapefruit (31 to 44), lemons (11 to 44), oranges (29 to 46), persimmons (43), and turnip roots (47.1). Sweet potatoes (20.3), limes (16 to 21), and tomatoes (17.9) contained appreciable amounts. Carrots, eggplant, grapes, onions, peaches, pears, plums, pomegranates, shallots, and watermelons contained less than 10 milligrams of ascorbic acid per 100 grams. Appreciable differences between different varieties of the same plant were indicated, such as from 2.7 to 9.1 milligrams per 100 grams in

nine varieties of watermelons, 29 to 46 in six varieties of oranges, 11 to 44 in seven varieties of lemons, and 7.3 to 37 in six varieties of cantaloupes. Cantaloupes, muskmelons, peaches, mustard, and persimmons contained more ascorbic acid than previously reported by other workers.

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INVESTIGATIONS OF THE CRACKING PROBLEM IN BRINING OF SWEET CHERRIES [1]

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For many years brined cherry stocks for eastern maraschino cherry processors were almost entirely imported from Italy and France. Only small quantities were barreled on the Pacific Coast for local use. Today that situation is almost entirely reversed. With the aid of a protective tariff on brined and processed cherries coming from Europe and with the more mechanical assistance of a standardized procedure of brining developed by Bullis and Wiegand (1931), the tonnage going into barrels in the western states has increased from 1,925 tons in 1925 to a high of 15,491 tons in 1936. This is almost a ten-fold increase in as many years.

The cherries barreled are almost entirely of the sweet varieties. The essentials of the brining process as used on the coast consist of filling standard 50-gallon, paraffin-lined, fir barrels with from 240 to 250 pounds of fresh cherries; covering these cherries with a solution known as brine; and with some agitation allowing the barrels to stand for four to six weeks or until the cherries are cured. The brine used is designed to both bleach and firm the cherries. It consists of a very dilute solution of sulphur dioxide and calcium carbonate or calcium hydroxide. The percentages recommended are 1.5 per cent sulphur dioxide and .9 per cent calcium carbonate or calcium hydroxide. In many localities the percentage is reduced below this figure with equally good results.

SOURCES OF SURFACE CHECKS

It is desirable, of course, to produce a bleached cherry of a light straw color, firm, and free from surface checks. Surface blemishes commonly found in brined cherries are of two general types: those formed by nature, such as rain checks, limb rubs, bird pecks, etc., and those caused by the solution or brine used. This latter type of blemish is commonly known as solution checks, and it was made the special object of this study. This deleterious solution checking occurs quite commonly among commercial packs. Its occurrence, however, in any one processor's pack is spotted. This observance alone

[1] These investigations were carried on through the coöperation of the Dalles Cooperative Growers and the Oregon Agricultural Experiment Station.

leads one to believe that the trouble is not caused entirely by varietal characteristics or a definite maturity factor but rather by the specific composition of the brine used—not only the percentage of materials used but also the relation of acid to alkali.

In commercial practice the skins of prunes are checked by a brief immersion in a dilute solution of either acid or alkali in preparation for drying. The similarity between this checking and the solution checks on brined cherries shows one quite definitely that the brine is the thing doing the checking; therefore, it is part of the problem to determine what brines check and what ones do not check.

A cherry is an organization of cells. Each cell has as a wall a semi-permeable membrane regulating the passage of material into and out of the cell. A relatively high concentration of sugars, starches, and gums is found inside the cell. The membrane will not normally allow these constituents to diffuse out readily but will allow water to diffuse into the cell to dilute the concentrated materials. By this theory when cherries are placed in a dilute brine, as they are in barreling, there would be an immediate tendency for the water to diffuse into the cherry until the concentration on each side of the cell wall would be equal. The tendency to build up pressure inside the cell would result in splitting of the tissue as a release of the pressure. Since the actual osmotic pressure of the bleach solution used is far below that of the contents of the cherry cell, one would expect that all the cherries would split, but actually this does not happen. As stated before, only parts of any one pack develop serious cracking trouble.

As a possible explanation of why this osmotic pressure does not develop, as a simple explanation of osmosis would lead one to believe, we note that Osterhout (1914) found that acid changes the cell membranes of plant tissues so that substances which would ordinarily be excluded to a greater extent can pass through more easily. This would indicate that the concentrated sugars, starches, and gums would, at least in part, be allowed to pass out through the membrane. Commercial observation indicates that this is actually quite probable in the cherry. If the cherries absorbed water only and there were no outward diffusion of the cell substances, the barreled fruit would be expected to gain weight in the barrels. Actually there is a loss of from 12 to 15 per cent in weight, indicating that the cell constituents do actually pass out through the membrane.

As further support to Osterhout and commercial experience, Stiles and Jorgensen (1917) found that tubers of potatoes and roots of carrots swelled less when immersed in tap water (a weak solution of a number of salts) than when immersed in distilled water. It is

evident that these salts, principally of calcium and magnesium, decreased the tendency to absorb water; or to satisfy the idea of osmosis, cell material passed out and water passed into the cell.

Although it is known that dilute acids alone will produce cracking in cherries, the problem is not so simple as just adding an excess of lime; for it has been shown that too much lime also produces violent cracking in cherries.

In previous work on this problem Cruess (1935) reports that cracking does not occur in brines having a pH of 2.25 to 3.7. Further it does occur in brines having a pH of 1.6 to 1.2. Although these results give some idea as to the effect of pH on cracking because of the inaccurate colormetric method of determining pH used, the exact figures given cannot yet be accepted as final. Of course, it should be understood that the pH figure is only a simple way of determining and indicating the acid-alkali ratio. The ratio can also be expressed in percentage of sulphur dioxide neutralized or the amount of each material used in the brine.

It therefore becomes our problem to determine the best acid-alkali ratio for the purpose of eliminating as nearly as possible the cracking of cherries. For the sake of simplicity it is also desirable to set this up in terms of pH.

EXPERIMENTAL PROCEDURE

Experiments were carried on with sweet cherries of the Royal Anne or Napoleon variety. A standard sulphur dioxide brine of 1.18 per cent was used. Four types of alkalies were used: commercial whiting (calcium carbonate), purified calcium hydroxide (powder), pure sodium hydroxide (50-per cent aqueous solution), and chemically pure potassium hydroxide (50-per cent aqueous solution).

The approximate percentages of sulphurous acid of the bleach solution neutralized in the tests were 12.5, 25, 37.5, 50, 62.5, 75, 87.5, and 100 per cent.

Two pounds of cherries were packed in two-quart jars and 960 c.c. of the solution was poured over them. The pH of the fresh solution and of the same solution six weeks after application were measured by means of a Goodhue-Dewitt glass electrode. At the end of the six-week storage period the cherries in each jar were counted for solution checks.

A second series was also started using the three varieties of cherries common to northwest barrelers—Royal Anne, Bing, and Black Republican. Instead of using the four different sources of neutralizing materials, only commercial whiting was used in this second series. In all other respects the conditions of the experiment were identical.

RESULTS AND DISCUSSION

The original brine to which no alkali was added had a pH value of 1.56, while that brine in which all the sulphur dioxide had been theoretically neutralized had a pH value of 2.12 (Table 1). One might expect this latter value to be nearer pH 7, but the sulphur dioxide solution is a weak acid and is able to resist quite strongly a

TABLE 1

pH Values of Freshly Prepared Solutions

Calculated SO_2 content neutralized	$CaCO_3$	$Ca(OH)_2$	KOH	NaOH
pct.				
12.5	1.61	1.61	1.63	1.64
25.0	1.66	1.64	1.66	1.68
37.5	1.76	1.69	1.76	1.74
50.0	1.80	1.78	1.80	1.80
62.5	1.88	1.85	1.93	1.91
75.0	1.96	1.90	1.96	1.98
87.5	2.08	1.96	2.08	2.12
100.0	2.17	2.12	2.13	2.24

change in pH, owing to its relatively large amount of undissociated hydrogen ions. As the solution comes very close to the neutral point, it takes little additional alkali to swing the pH over on the basic side.

TABLE 2

Final pH of Solutions—Royal Anne Series

(After six weeks' storage)

Calculated SO_2 content neutralized	$CaCO_3$	$Ca(OH)_2$	NaOH	KOH
pct.				
12.5	2.59	2.66	2.54	2.59
25.0	2.81	2.84	2.86	2.78
37.5	3.22	3.12	3.30	3.08
50.0	3.54	3.44	3.62	3.39
62.5	3.91	3.72	4.15	3.64
75.0	3.99	3.98	4.60	3.88
87.5	4.03	4.05	5.20	4.23
100.0	4.23	4.13	5.59	4.65

The data showing the pH values after six weeks' storage indicate that in each case the value has increased and is nearer the neutral point (Table 2).

The percentages of perfect cherries in relation to the pH of the solution and the form of alkali used to adjust the pH are shown (Table 3). The cracking curves for whiting and for hydrated lime

show the maximum percentage of perfect cherries at very nearly the same pH value, that is, pH 1.96 for whiting and pH 1.94 for hydrated lime (Fig. 1). The maximum point for sodium hydroxide is

TABLE 3

Percentages of Perfect Cherries—Royal Anne Series

Calculated SO_2 content neutralized	$CaCO_3$	$Ca(OH)_2$	NaOH	KOH
pct.				
12.5	7.8	3.4	1.8	5.3
25.0	19.0	10.4	3.5	15.3
37.5	46.0	9.5	8.0	27.4
50.0	65.6	28.3	13.6	20.5
62.5	76.4	48.8	32.5	35.7
75.0	87.6	51.3	52.2	32.4
87.5	61.5	43.1	55.3	63.0
100.0	40.8	46.5	35.9	77.5

reached at pH 2.07 and for potassium hydroxide is above pH 2.13. There is reason to believe that the potassium hydroxide curve reaches a maximum not far beyond pH 2.13, the last point plotted on the curve, and breaks downward with increased cracking injury.

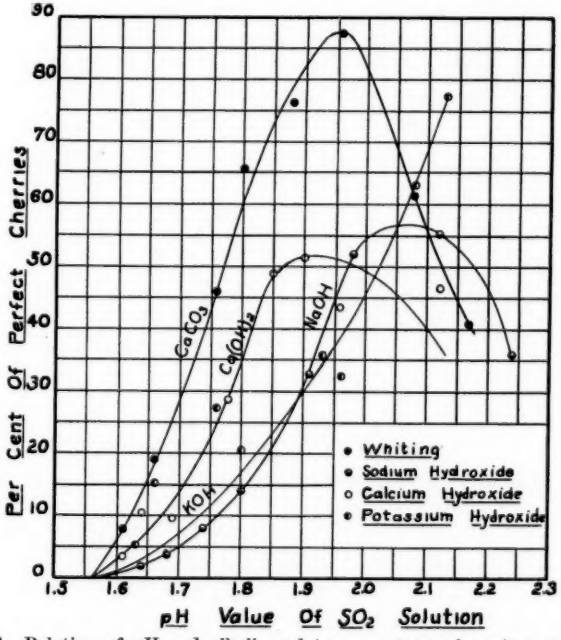

FIG. 1. Relation of pH and alkali used to percentage of perfect cherries obtained in brining.

The surprising feature noted in comparing the curves for whiting and hydrated lime is the greater number of perfect cherries in the whiting series at the same pH value. A possible explanation is that there may be some effect exerted by the small amount of carbonic acid in the whiting series. The carbon dioxide released when the

TABLE 4

Percentages of Perfect Cherries—$CaCO_3$ Series

pH of original solution	Royal Anne	Bing	Black Republican
1.56	None	None	22.6
1.61	7.8	No test	No test
1.66	19.0	None	58.0
1.76	46.0	7.5	83.4
1.80	65.6	23.5	98.1
1.88	76.4	17.2	97.9
1.96	87.6	3.45	92.9
2.08	61.5	None	90.4
2.17	40.8	None	90.7

carbonate is dissolved in the brine produces carbonic acid. Most of the carbon dioxide escapes, but some undoubtedly remains in solution.

Data on the percentages of perfect cherries of each variety with respect to the pH value of the brine solution employed (Table 4 and Fig. 2) show that the results on the Bing cherries are abnormally

TABLE 5

Final pH Values After Six Weeks' Storage—$CaCO_3$ Series

Calculated SO_2 content neutralized	Royal Anne	Bing	Black Republican
pct.			
12.5	2.59	.....	.....
25.0	2.81	2.81	3.23
37.5	3.22	3.22	3.47
50.0	3.54	3.59	3.83
62.5	3.91	4.03	4.16
75.0	3.99	4.03	4.16
87.5	4.03	4.18	4.25
100.0	4.23	4.28	4.47

low and do not represent what is actually obtained in commercial practice, but they show that the Bing is more delicate than the other varieties and that its optimum pH value is different from that of the Royal Anne cherry. The final pH value after six weeks' storage is shown (Table 5).

CONCLUSIONS

It is apparent from the experiment with Royal Anne cherries that the pH value of brine solutions is a factor in preventing cracking of cherries. It is also apparent that the type of alkali used in the solution is an important factor; for while a pH value of 1.94 is suitable when calcium is used, it is not suitable when sodium or potassium is employed. These latter alkalies require definite pH values which

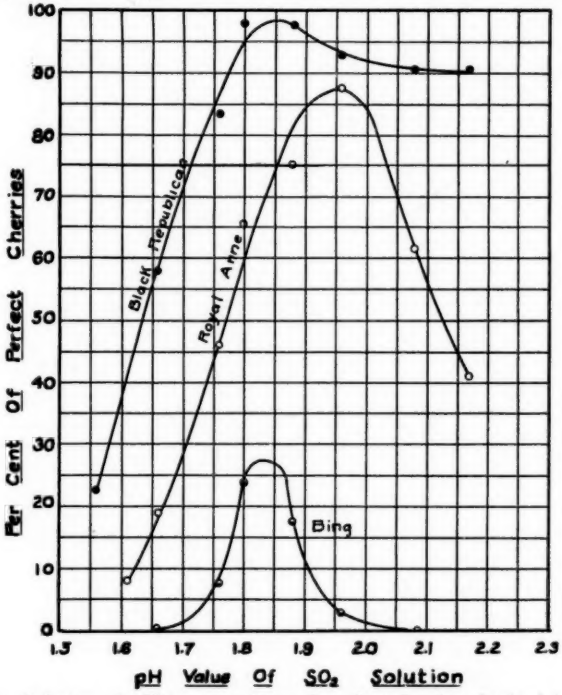

FIG. 2. Relation of pH to percentage of perfect cherries obtained in brining of three common varieties.

are higher than is suitable for calcium. Cruess (1935) estimated the critical pH value (point at which least cracking occurs) to be between pH 2.25 and pH 3 regardless of the type of alkali used to adjust the pH. This estimate appears to be too high for calcium-sulfur dioxide solutions. The optimum pH value of solution for Royal Anne cherries was found to be lower, namely pH 1.95.

The results of the experiments with black cherries indicate that the variety of cherry has a bearing upon the optimum pH value which should be employed. The indication is that a solution of one

pH value should be used for Royal Anne cherries and one solution with a lower pH value for Bings and Black Republicans. The optimum hydrogen ion concentration of solutions for black cherries was determined at pH 1.83.

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USE OF STABILIZERS IN UNAGITATED ICE CREAMS

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In spite of the increased number and growing use of refrigerators in the home, very little experimental work on refrigerator ice creams has been published.

The chief differences between refrigerator and other ice creams are due to the fact that the latter are agitated during freezing while the former are not. The agitation has three main functions: (1) incorporation of air, (2) prevention of ice crystal growth, resulting in a smoother product, and (3) prevention of the formation of a cream layer during freezing in cases where the mix has not been homogenized. In refrigerator ice creams the air may be incorporated by whipping the cream or other foaming agent and folding it into the mix before freezing; crystal growth is customarily prevented, both in the prepared mixes and in current recipes, by the use of a very high percentage of butter fat generally in combination with a stabilizing agent; and separation of a cream layer is prevented by using either a stabilizing agent or a still higher percentage of butter fat. These high percentages of fat make the cost high and the caloric value excessive. The object of the present research has been to find a method of producing an unagitated ice cream having both a desirable body and texture and a low fat content.

EXPERIMENTAL PROCEDURE

The ice creams were made up in small amounts and frozen in small, straight-sided, metal gelatin molds (capacity two ounces) in the freezing compartment of a refrigerator. The temperature varied from —9.5 to —15°C. (15 to 5°F.) but this range was not thought to be greater than the average variation in different refrigerators and in the same refrigerator at different times. The average temperature during freezing was —11.7°C. (11°F.). Average temperature at the time of removal of the samples was —12.4°C. (9.5°F.).

Whipped cream folded into the mix was the means of incorporating air in the ice creams. Skim milk was used in the ice cream mix in order to make the amount of cream to be whipped as large as possible without increasing the fat content of the ice cream. The cream was weighed after whipping, as it was impossible to whip the amounts used separately without undue losses. Two teaspoonfuls of vanilla

were added for each cup of cream after it was whipped in order to keep the amount of vanilla used constant. The skim milk was measured in a graduated cylinder and all materials were weighed on a trip balance. For weighings of one gram or less an analytical balance was used.

The following basic recipe was worked out and used as a control: 37.5 c.c. skim milk, 16.6 gm. cream, 9.4 gm. sugar, vanilla. The basic recipe is compared (Table 1) with the composition of the average commercial mix as given by Sommers (1935).

All ingredients except the cream and flavoring were combined, the method of mixing depending upon the stabilizer used. The whipped cream and flavoring were then folded into the mix and

TABLE 1

A Comparison of Percentage Compositions of Basic and Commercial Mixes

Ingredients	Basic recipe	Commercial ice creams		
		Average	High	Low
	pct.	*pct.*	*pct.*	*pct.*
Fat	10	12.5	22	8
Serum solids	6.5	10	12	6
Sugar	14	16	17	12

the combined mix was frozen in the refrigerator, which had been kept at its fastest freezing point for at least one hour.

Rennet

A rennet solution was made by dissolving one junket tablet in 12 c.c. of water. A firm, soft curd was produced in 37.5 c.c. of milk with 3.4 c.c. of the rennet solution. Ice creams were made using .5, 1, 3.4, and 4.5 c.c. of the rennet solution. A control was made, using an added equivalent amount of water. The milk was warmed to 40°C.(104°F.), the sugar was stirred into the milk, and the rennet solution was added. This mixture was then placed in the refrigerator and frozen to a mush. The whipped cream and flavoring were folded in and the ice cream completely frozen.

The rennet-treated samples could not be differentiated from the control in texture or body. With increasing amounts of rennet there was an increase in a salt-like flavor of the ice cream. The texture in all of the samples was flaky with very large ice crystals. When unstirred, the cream rose and the ice cream separated into two layers. An increase in the serum-solids content improved the texture but again the product without rennet was similar to the product which contained rennet.

Milk was coagulated with rennet and frozen. At the same time a sample of untreated milk was frozen. Moderately large crystals were produced throughout the milk, while in the rennet-clotted sample the crystals showed a greater tendency toward large flakes and spines. The untreated milk melted to its normal state. On melting the clotted milk contained a small shrunken clot in the center and a band of clear liquid at the outer edge. Rennet apparently coagulates the milk, but the water is not held firmly and on freezing is squeezed out of the paracasein clot and produces a separate crystalline phase.

Rennet appears to have no stabilizing effect in refrigerator ice creams.

TABLE 2

Ice Creams Containing Varying Amounts of Serum Solids

Sample	Flavor	Texture	Body
1. Control (no dried milk), 6.4% serum solids	Vanilla	Coarse, very large crystals	Liquid separated in two layers
2. Base plus 2.4 gm. dried milk, 10% serum solids	Vanilla, somewhat salty	Coarse, very large crystals	Liquid separated in two layers
3. Base plus 3.8 gm. dried milk, 12% serum solids	Vanilla, increased rich, salt-like flavor	Coarse, large crystals	Thin
4. Base plus 5.8 gm. dried milk, 15% serum solids	Vanilla, rich, salty	Somewhat less coarse, medium-large crystals	Very slightly viscous

Serum Solids

The serum-solids content of commercial ice creams is generally increased by the addition of dried skim milk and condensed milks. The results obtained by varying the serum-solids content of refrigerator ice creams by the addition of dried skim milk show that a serum-solids content of 10 per cent did not prevent a floating of the cream layer (Table 2). Though a serum-solids content of 12 per cent or more prevents separation of the cream in stirred ice creams, serum solids alone cannot be considered an efficient stabilizer because the texture was still very coarse. Increasing the percentage of serum solids increases the stability of the ice creams.

Pectin

Grade-100 citrus and grade-100 apple pectin [1] were used in refrigerator ice creams. The pectin was mixed with the sugar and then

[1] The grade of pectin refers to the number of parts of sugar which can be jelled by one part of the pectin. Grade-100 pectin will jell 100 times its weight of sugar.

combined with the milk. The pectin was brought into solution by heating the mixture to 85°C.(185°F.). A curd was produced on heating if the milk used was not strictly fresh, and unless the mix was cooled at once a curd resulted. The cooled mix was chilled in the refrigerator until it began to freeze to the sides of the molds. At that point the whipped cream and flavoring were folded in and the ice cream was frozen without stirring. A summary of the ice creams produced with varying amounts of pectin (Table 3) shows that one

TABLE 3

Pectin Ice Creams (Grade 100)

Sample	Flavor	Texture	Body
1. Control	Strong vanilla	Coarse, very large, flaky crystals	Thin, separated in two phases
2. .4% pectin	Vanilla	Coarse, a few very large, flaky crystals	Thin, separated
3. .8% pectin	Vanilla flavor somewhat masked	Large crystals, ice cream slightly less coarse	Slightly viscous
4. 1% pectin	Pectin-like	Fine texture, small crystals which can be detected	Viscous
5. 1.2% pectin	Pectin	Fine texture, small crystals which can be detected	Viscous
6. 1.4% pectin	Pectin	Fine texture, small crystals which can be detected	More viscous
7. 1.8% pectin	Strong pectin	Very smooth	Heavy, viscous, fine particles

per cent of grade-100 citrus or apple pectin was found to produce a comparatively smooth ice cream with a body that was viscous but not objectionably so. Pectin had a marked tendency to mask flavor and sweetness. A better flavor was obtained by increasing the amount of sugar from 9.4 to 12 grams and doubling the amount of vanilla. Pectin-vanilla ice creams have a flavor typical of pectin. This alteration of flavor was relished by some people but not by everyone. Apple pectin gave the ice cream a tan color and a flavor suggestive of maple. It was found to be more suitable in maple-flavored ice creams. The use of citrus pectin in this maple ice cream resulted in an inferior flavor. A citrus pectin of higher gel strength (grade 172) when used in proportionately lower concentrations produced no detectable differences in the body and texture of the ice creams, however, there

was an unexpected flavor difference. The high-grade pectin ice cream had a less pectin-like flavor. The manufacturer explained this flavor difference as being due to the method of processing the pectin.

Agar

Agar gels do not reform after they are broken. Agar ice creams when stirred or churned have a tendency to become crumbly, Dahlberg, Carpenter, and Hening (1928). Agar was satisfactory in refrigerator ice creams because agitation was unnecessary.

A granular, purified variety of agar without noticeable taste or odor was used in these experiments. The agar was mixed with the sugar and milk and boiled. A smooth ice cream was produced with .4 to .6 per cent agar (Table 4); however, these ice creams had a

TABLE 4

Agar in Refrigerator Ice Creams

Sample	Texture	Body
1. Control	Coarse, very large, flaky crystals	Thin, separated in two phases
2. .2% agar	Coarse, medium crystals	Slightly viscous
3. .4% agar	Fine texture, small detectable crystals	Spongy on melting
4. .6% agar	Smooth, crystals not detectable	Slightly crumbly, spongy on melting
5. .8% agar	Very smooth, no ice crystals formed	Gel, no melting until very high temperature

high melting resistance and a spongy body on melting. When the concentration of agar was as high as .8 per cent no ice crystals were produced at a temperature of —13.5°C.(8°F.). The resulting product was similar to a Spanish cream or a pudding. When the amount of agar was reduced to .2 per cent the body was normal but the texture was no longer fine. It was found that increasing the serum-solids content improved the texture of the .2-per cent agar ice cream without impairing the body or unduly increasing the melting resistance. A serum-solids content of 12 to 15 per cent was satisfactory. These body and melting effects were clearly demonstrated by placing the ice creams on quarter-inch wire mesh, over funnels and graduates, as noted by Cantfield and Martin (1933). Both the relative amount of drain and the type of drain furnished a basis for body comparisons. At the end of one hour the .2-per cent ice cream, which contained additional serum solids and a commercial ice cream used for

comparison, had practically melted. Ice creams containing .4 and .6 per cent of agar had a jelly-like consistency and showed a very small amount of drain. This drain was not the true ice cream mix but was a thin, milky whey.

Tiny agar particles could be found in the ice creams on melting. This was still true when the agar-milk mixture was boiled one minute; however, when a finely powdered form of agar was used it went into solution more readily on boiling and the agar particles did not result.

Gelatin

Gelatin has long been used in commercial ice creams. Its beneficial effect is due to the formation of a gel, according to Dahlberg, Carpenter, and Hening (1928).

TABLE 5

Varying Concentrations of Gelatin in Refrigerator Ice Creams

Sample	Texture	Body
1. Control	Coarse, very large, flaky crystals	Thin, separated in two phases
2. .2% gelatin	Coarse, very large flaky crystals	Thin, separated in two phases
3. .4% gelatin	Coarse, medium-large crystals	Thin, separated in two phases
4. .6% gelatin	Slightly coarse, medium-large crystals	Viscous, not separated
5. .8% gelatin	Slightly coarse, medium-large crystals	Somewhat spongy
6. 1% gelatin	Slightly coarse, slightly fewer medium-large crystals	Spongy

Gelatin in varying concentrations was added to the basic recipe. The dry ingredients were mixed and the skim milk was stirred in. The mixture was heated to 60°C.(140°F.) to dissolve the gelatin. The mixture was then cooled and finally chilled in the refrigerator until it started to freeze on the sides of the container. The cream and flavoring were then folded in and the ice cream frozen. Gelatin had a definite stabilizing effect when the concentration was .6 per cent or over (Table 5). No improvement in texture was apparent and the body was impaired when higher concentrations were used. Ice creams containing .6 per cent of gelatin as a stabilizer were fairly coarse. Increasing the serum-solids content improved the gelatin ice creams; the best ice cream was produced with 15 per cent serum solids. The product was not as smooth as the ice creams produced with agar and pectin.

India Gum

An attempt was made to find an efficient stabilizer which would go into cold solution. India gum was used in concentrations varying from .2 to 1.4 per cent. A concentration of .8 per cent had a definite stabilizing effect and prevented a rising of the cream. Difficulty was experienced in getting the gum to go into the solution without lumping. By mixing it with powdered sugar and using a rotary beater a finer separation was effected. All the ice creams contained swollen particles of gum which were not detectable in the mouth. The particles gave the ice cream an undesirable curdled appearance on melting. Boiling the solution increased the swelling of the gum and

TABLE 6

Egg Yolk in Combination With Other Stabilizers

Sample	Flavor	Texture	Body
1. 5 gm. egg yolk	Nice, vanilla	Coarse, large crystals	Thin, not separated
2. 5 gm. egg yolk plus 6 gm. dried skim milk	Undesirable, somewhat similar to evaporated milk	Less coarse, medium crystals	Viscous
3. 5 gm. egg yolk plus 1.5 gm. starch (corn)	Good, slightly starchy	Improved texture, medium-small crystals	Custard-like, heat resistant, heavy body
4. 5 gm. egg yolk plus 1.93 gm. starch (wheat)	Good, slightly starchy	Improved texture, medium-small crystals	Custard-like, heat resistant, heavy body
5. 5 gm. egg yolk plus .68 gm. pectin (1% of grade 100)	More decidedly pectin than pectin alone	Improved texture, medium-small crystals	Viscous but thinner than pectin alone

therefore increased the smoothness of the ice cream. However, the particles could still be seen on melting. Since smooth ice creams could be produced with other stabilizers when heated and no particles would be apparent in the melted product, India gum was not considered a satisfactory stabilizer.

Egg Yolk

Egg yolk was used alone and in combination with various other stabilizers. The mixes were heated in a boiling water bath and cooled before the cream and flavoring were folded in. Egg alone produced a coarse product (Table 6). The mix was stable enough to prevent separation. Dried skim milk improved the texture but the flavor combination was undesirable. In combination with the two types of starch the melting resistance was high and the body heavy.

Egg yolk when used with pectin did not improve the texture and resulted in an inferior flavor.

Cornstarch

Both corn and wheat starch were used in varying percentages. Wheat starch acted just like a lower concentration of cornstarch. The ice creams were somewhat similar to pectin ice creams but cornstarch was not as effective as pectin in reducing the size of the crystals. Cornstarch masked the flavor in much the same way that pectin did but had no flavor of its own. When cornstarch was used there was a tendency for the ice creams to freeze harder. When a serum-solids content of 15 per cent was used, a pasty body resulted. The best starch ice cream was produced by using two per cent of starch and additional dried skim milk to bring the serum-solids content up to 12 per cent.

Chocolate

Chocolate was used in pectin ice creams to investigate its possible stabilizing effect. The use of chocolate improved the texture of the ice cream. On further experimentation this improvement was shown to be a result of the increased solids content rather than any one specific factor, such as the cocoa butter. The same improvement in texture was produced by increasing the sugar in equivalent amounts. That increased sugar results in an improvement in texture has been pointed out by Dahlberg (1925). Cocoa solids were not as effective as sugar but acted more like starch, making the product freeze harder. Chocolate, when used with one per cent of citrus pectin, produced a satisfactory ice cream. When apple pectin was used, the flavor combination was undesirable, the chocolate flavor being impaired.

Corn Sirup

The use of corn sirup in refrigerator ice creams has often been recommended. The texture of the samples made was improved by substituting corn sirup for one-fourth to one-half of the sugar. (For equal sweetness it is necessary to replace one part by weight of sugar with 3.2 parts by weight of corn sirup.) A substitution of corn sirup for three-fourths of the sugar resulted in a soft ice cream, owing to lowering of the freezing point.

DISCUSSION

In considering the role of stabilizers in refrigerator ice creams it is found that much of the work done in the commercial field with stabilizers does not apply, owing to the absence of agitation. Agitation affects the action of certain stabilizers. This difference is apparent when agar is used as a stabilizer.

The work of Dahlberg, Carpenter, and Hening (1928) shows that the principal property of gelatin in ice creams is its ability to form a gel. Gelatin possesses the ability to reform a gel structure in ice cream after the gel has been destroyed by agitation. This property is not exhibited by agar gels. Agar does not prevent a crumbly body in churned ice cream because it does not form a gel in the frozen product. When used in refrigerator ice creams an agar gel does form and the body is not crumbly unless the ice cream is stirred.

Whitaker (1929) indicates that owing to agitation there is a reduction to a basic viscosity and that this basic viscosity, rather than the apparent viscosity which is ordinarily measured, is an important factor in commercial ice creams. In refrigerator ice creams the viscosity remains unaltered owing to the absence of agitation.

It is hoped that the stabilizers which have been found most effective will be put on the consumers' market in suitable grades and packages. Since only small amounts are used, they should not be expensive.

The following recipes for the various stabilizers have been developed from the basic experimental work. All of the ice creams except those in which agar is used can be stirred, if so desired, to facilitate freezing. Freezing is facilitated by scraping the frozen part from the sides of the tray, especially when the tray is large; stirring, however, is not required. These ice creams are frozen most satisfactorily in refrigerators which have the quick-freeze control. They can be frozen in the other models if the temperature is sufficiently low. The texture will be coarsened somewhat by the slow rate of freezing. The temperature should be as constant as possible to prevent crystal growth, Cole (1932). When the ice cream has frozen, the control should be turned down to a temperature proper for holding.

RECIPES

Vanilla, agar

2 c. skim milk,
⅔ c. sugar,
1 c. whipping cream (38%),
½ c. dried skim milk,
2 tsp. vanilla,
1 tsp. agar.

Mix sugar, dried milk, and agar thoroughly; stir in the milk and boil one minute. Allow to cool until lukewarm. (Agar gels set at 37.5°C.(99.3°F.). After gelation the cream will not combine smoothly.) Stir in the cream (best whipped in a chilled bowl) and flavoring and freeze. Approximate freezing time two hours.

Vanilla, pectin

2 c. skim milk,
⅝ c. sugar,
1 c. whipping cream,
2½ tsp. citrus pectin, grade 172 (or 3 tsp. of grade-100 citrus pectin),
4 tsp. vanilla.

Mix dry ingredients thoroughly and stir in the milk. Heat in a double boiler for ten minutes (by quick method—bring just to the boil, stirring constantly). Cool immediately. Chill in the refrigerator until it starts to freeze onto the sides of the tray. Fold in the whipped cream and flavoring. Freeze.

Vanilla, gelatin

2 c. skim milk,
2 tsp. gelatin (5.7 gm.),
1 c. whipping cream,
⅔ c. sugar,
2 tsp. vanilla,
½ c. dried skim milk.

Mix dry ingredients, add milk, and heat to dissolve gelatin. Cool, chill as for pectin, and fold in whipped cream and flavoring. Freeze.

Vanilla, cornstarch

2 c. skim milk,
⅔ c. sugar,
2 tbsp., ½ tsp. cornstarch,
⅜ c. dried skim milk,
1 c. whipping cream,
3 tsp. vanilla.

Mix dry materials, add milk, and heat until the starch thickens. Cool thoroughly and fold in the whipped cream and flavoring. Freeze.

Maple, apple pectin

2 c. skim milk,
⅝ c. brown sugar,
1 tbsp. apple pectin (grade 100),
1 c. whipping cream,
2 tsp. mapleine.

Use same method as that for vanilla pectin.

Chocolate, citrus pectin

2 c. skim milk,
⅞ c. sugar,
1½ sq. bitter chocolate,
2½ tsp. citrus pectin (grade 172),
1 c. whipping cream,
2 tsp. vanilla.

Melt chocolate and stir into the hot milk mixture; beat with rotary beater until smooth. Cool and proceed as for vanilla pectin.

CONCLUSIONS

1. Rennet is not an effective stabilizer in refrigerator ice creams.

2. Dried skim milk, chocolate, corn sirup, and increased sugar have some stabilizing action but the concentrations in which they can be used are limited.

3. Egg yolk and India gum have stabilizing action, but possess other characteristics which are undesirable.

4. Agar, when used in combination with dried skim milk, produces the best refrigerator ice cream.

5. Pectin produces an ice cream of desirable texture although it has a more viscous body and a greater melting resistance than the agar ice cream. Pectin ice creams also have a typical flavor. On the whole, pectin ice creams were considered satisfactory. The use of dried skim milk with pectin was not considered desirable or necessary.

6. Gelatin ice creams with dried skim milk have a coarser texture than do pectin or agar ice creams. The body and melting are quite normal.

7. When cornstarch and serum solids are used as the stabilizing agent the ice creams freeze harder, the body is somewhat viscous, and there is a slight masking of flavor.

8. The texture of the ice creams produced is not identical with that of commercial ice creams. A refrigerator ice cream cannot be made as ultimately smooth as a churned ice cream with this low percentage of fat; however, the ice creams produced are readily acceptable and very palatable.

Note: This work has also been extended to sherbets and frozen fruit salads. Of the stabilizers found successful in ice creams, agar produced the best sherbet, gelatin and pectin being detrimental to the fine texture and light body of sherbets. Agar or gelatin combined with mayonnaise proved satisfactory in frozen fruit salads. Pectin, in the dry state, could not be combined in a satisfactory manner where no sugar was added to separate the pectin particles and so prevent lumping.

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PECTIC ENZYMES. III. HEAT INACTIVATION OF TOMATO PECTIN-METHOXYLASE (PECTASE)[1]

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In order to obtain a product of good consistency it is essential to preserve during manufacturing the pectin naturally occurring in tomatoes. As shown in a previous article by the author (1938), tomatoes contain among other enzymes a very active pectin-methoxylase (pectase). When in the course of preparation the cell structure of the tomatoes is destroyed, this enzyme gains access to the pectin and by splitting off methyl alcohol transforms it into pectic acid. This latter compound does not possess the important colloidal properties of pectin and thus a degradation in the consistency of the product occurs.

The enzymic character of the changes which take place in crushed tomatoes has long been assumed but no information was available regarding the chemical reactions involved. In the course of manufacturing tomato juice, the fruit is usually heated before or immediately after extraction. The amount of heat applied has been empirically determined by its effect on the quality of the product. With the observation that tomatoes contain a very active pectin-methoxylase but no pectin-polygalacturonase, as noted by Kertesz (1938), the nature of the most important reaction was clarified. Of course, other changes may also occur in crushed tomatoes, but the demethoxylation of pectin is undoubtedly the most important of them from the standpoint of consistency at least.

Since heating is at the present time the only practical means available for inactivation of the pectin-methoxylase in tomatoes, a study of the sensitivity of this enzyme towards heat was desirable. It was also important to determine the minimum amount of heat which will cause a complete inactivation of the enzyme. In the present paper the results of experiments on these questions are presented and their significance discussed.

EXPERIMENTAL PROCEDURE

For these experiments material homogeneous in its enzyme content was needed. For this reason not whole tomatoes but rather the

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juice pressed from them by hand was used. Usually only a small quantity of seeds and skin remained on the cloth after pressing. The juice was once more strained through cloth and kept at 5°C. (41°F.) under toluol. For the determination of the enzyme activity the method described in an earlier publication by Kertesz (1937) was used. The tomato-juice samples contained 80-110 pectin-methoxylase units per gram of dry matter and in the neighborhood of five per cent of total solids.

For the inactivation one-cc. samples of the juice were pipetted into thin-walled test tubes. These were immerged into water of the desired temperature for the required period and then immediately cooled in cold water. In order to avoid any change in the temperature caused by the immersion of the cold test tube, a bath containing over one liter of water was used. After a few minutes of cooling the content of the test tube was mixed with pectin and the enzyme activity determined (Fig. 1) by the said method at pH 6.2 and 30°C. (86°F.).

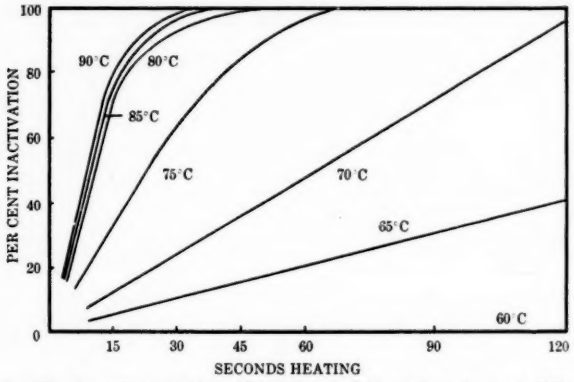

FIG. 1. The heat inactivation of tomato pectin-methoxylase at different temperatures.

Heating the juice at 60°C.(140°F.) for two minutes had no effect on its enzyme activity determined subsequently. At 65°C.(149°F.) about 40 per cent of the activity was lost in two minutes. Thus the "critical inactivation temperature" of this enzyme (at which 50 per cent activity is lost in one hour) is between 60 and 65°C.

The curves (Fig. 1) need little explanation. The greatest increase in the rate of inactivation per degree rise of temperature occurs between 70 and 75°C.(158 and 167°F.). At 80°C.(176°F.) the rate of inactivation seems to reach a maximum and is but little altered by using higher temperatures. Naturally in case of such short peri-

ods of heating the time required to warm up the sample from room temperature should also be considered. In a set of experiments tomato juice was warmed to 60°C. before the inactivation at 80°C. was started. The curve showing the progress of inactivation was but slightly steeper than that obtained with cold juice (Fig. 1). Complete inactivation was reached in these determinations in about 35 seconds as contrasted with the 45 seconds needed in case of cold juice. But holding the juice at 60°C. before the actual inactivation is started may affect the flavor. At this temperature the reactions catalyzed by the enzyme will also proceed several times as rapidly as in the juice at room temperature. Thus from the practical standpoint the warming of the juice prior to inactivation is not desirable.

The presence of other materials besides the enzyme in the juice as well as its reaction (pH) have an influence on the rate of inactivation. Since the pH of ripe tomatoes shows little variation, this factor will probably not cause appreciable divergence from the rates given (Fig. 1). On the other hand there may be considerable variation in the composition and total solids content of different tomatoes. When a large number of samples prepared from tomatoes of different types and varieties were heated to 80°C.(176°F.) for 45 seconds, the enzyme was always completely inactivated. To observe the inactivation under extreme conditions some tomato juice was centrifuged and the sediment was heated as the above samples were. Only two per cent of the original enzyme activity could be found in the subsequent determination.

It is difficult to measure the temperature of the small volume of tomato juice during a period of less than a minute. The temperature in the present experiments has been measured in the bath only and a few seconds must have passed until the juice itself reached the inactivation temperature. Exactly 45 seconds were needed for the inactivation of the enzyme at 80°C. It is safe to conclude that 45 seconds of heating to 80°C. when the temperature is measured in the juice will always completely inactivate the pectin-methoxylase in tomato juice. In case the heating is performed in tubes in which the juice is flowing or in the presence of seeds or with the pulp not entirely broken up, heating for a slightly longer period may be advisable. Heating to higher temperatures may have an undesirable effect on the flavor without increasing the rate of inactivation appreciably.

The effectiveness of the heating under commercial conditions can be easily controlled. A one-cc. sample of the juice should be added to 25 cc. of only slightly acid pectin solution and the mixture titrated with .1N alkali in the presence of methyl red until the mixture loses

its last pink tint from one drop of alkali. If the pectin-methoxylase is completely inactivated, the mixture should show no change of color at room temperature in an hour.

SUMMARY

The pectin-methoxylase (pectase) of tomatoes is completely inactivated by heating the juice to 80°C.(176°F.) for 45 seconds. The completeness of the inactivation can be easily determined by a simple test.

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Since the submission of this paper for publication the investigations dealing with the thermal inactivation of pectin methoxylase have been extended. The author feels that the addition to the text of one table concerning the effect of actual acidity (pH) on the time required for complete inactivation of the enzyme will be of interest. The inactivation of the enzyme was carried out as in the experiments of the above paper and the residual activity determined by the proposed qualitative method. Mr. James C. Moyer collaborated in these determinations.

Pectin-methoxylase in enzyme solutions heated for two minutes at pH	6.0	4.4	2.5	1.1
Temperature				
47°C.(117°F.)	+	+	+	+
50°C.(123°F.)	+	+	+	—
55°C.(132°F.)	+	+	—	—
65°C.(150°F.)	+	+	—	—
70°C.(159°F.)	+	(+)	—	—

It appears that as the pH is lowered below 4.4 the sensitivity of the enzyme towards heat is increased a great deal.

A METHOD FOR STUDYING THE HISTOLOGICAL STRUCTURE OF FROZEN PRODUCTS. I. POULTRY

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Numerous authors have been concerned with problems relevant to the histological structure of frozen tissues. Their principal interest has been to ascertain where ice forms with respect to constituent muscle fibers when the tissues are subjected to various freezing and storage temperatures and to determine to what extent freezing alters the structure and properties of such tissues. In preparing frozen tissues for microscopic study, it has been the usual procedure to use fixing fluids chilled to sub-zero temperatures. Cook *et al.* (1926), in studies on the refrigeration of meat, for example, fixed frozen tissues by dropping small pieces into a 10-per cent formalin solution which had been cooled to its freezing point. Subsequently, the tissues were removed from the sub-zero temperature and sections were prepared in the conventional manner for microscopic examination. Another method commonly employed has been to cut very thin, free-hand sections of frozen tissue and to examine them microscopically while in the frozen condition. This method obviously reveals very little concerning the exact location, in terms of constituent muscle fibers, of the ice formations, and histologically it is of little value.

Recently a number of problems have arisen that necessitate a histological study of frozen muscle tissue. Considerable difficulty has been encountered in securing perfect fixation of the frozen tissues when employing fluid fixatives. It has been found, when using formalin and even more penetrating fixatives, that the tissues and protoplasm surrounding the ice formations were not always thoroughly fixed and hence upon removal to room temperatures and subsequent thawing, semblances to the frozen condition would be obliterated. Failure to secure adequate fixation of frozen tissues when using chilled-fluid fixatives is due to the difficulty that these fluids have in penetrating even very small pieces of frozen tissue.

It appeared that the histological and cytological structure of the tissues in frozen-food products could be more successfully studied by adopting a method which in principle would be similar to the Altmann-Gersh freezing-drying method. Essentially this technique consists of freezing the tissues rapidly—immersing in pentane chilled to —131°C.(—203.8°F.) or iso-pentane chilled to —195°C.(—319°F.),

etc.—and then placing them in a vacuum-dehydrating chamber cooled to sub-zero temperatures until dehydration has been accomplished. Subsequent treatment of tissues may follow the more conventional techniques. This method introduced by Altmann (1890) and elaborated by Gersh (1932) is now being used successfully by Hoerr (1936) and others in studying biological problems. It is advantageous in studying certain cytological and histochemical problems, in that fixation by rapid freezing is almost instantaneous and hence causes almost immediate cessation of cellular activity. The procedure discourages the redistribution of cellular components and preserves cellular constituents, in that dehydration *in vacuo* eliminates the necessity of subjecting the tissues to strong solvents.

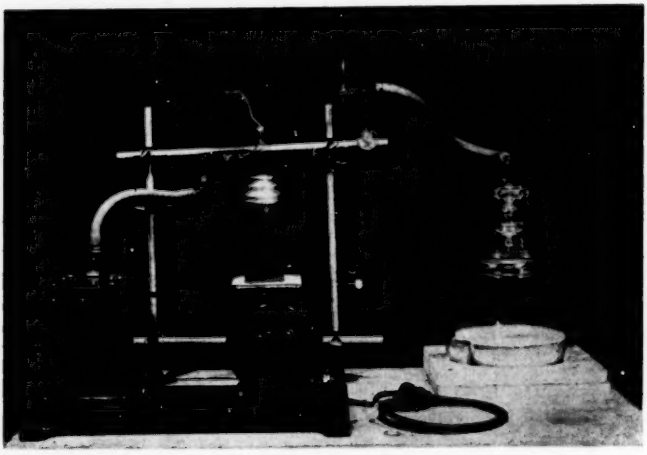

FIG. 1. Vacuum-dehydrating apparatus.

As applied to the study of the structure of tissues from frozen-food products, we are chiefly interested in that part of the Altmann-Gersh method pertinent to the dehydration and fixation of frozen tissues.

EXPERIMENTAL PROCEDURE

Where freezers are a part of the laboratory equipment, it is not a difficult problem to construct an apparatus that will remove the water from the tissues of frozen products and simultaneously fix the tissues by drying. The problem consists of constructing an efficient vacuum-dehydrating apparatus which during the period of functioning remains in sub-zero temperatures. The apparatus (Fig. 1) which has been found entirely satisfactory consists of a Cenco-Hyvac pump

run by a one-eighth horse-power electric motor connected to a Pyrex desiccator serving as the dehydrating chamber. A light oil is used in the pump and motor since the apparatus operates in a —35.6°C. (—32°F.) freezer, and the vaseline used in sealing the stopcocks and desiccator is cut with kerosene.

Small samples of tissue taken from the frozen products are placed in the desiccator, and when the vacuum is established, the vapor pressure of the ice is greater than the vapor pressure in the dehydrating chamber; consequently, the water will leave the frozen tissue. This vaporized water is picked up by anhydrous phosphorus pentoxide placed in the bottom of the desiccator. A very small amount of water will escape being picked up by the phosphorus pentoxide and in order to prevent it condensing in the vacuum pump, two traps are inserted in the system. Dry ice contained in a thermos bottle placed about one or both of these traps will tend to freeze out the moisture before it gains access to the pump.

A stopcock is interposed between the oil pump and the dehydrating chamber and permits breaking of the vacuum at the completion of dehydration. A stopcock is also placed in the system immediately above the dehydrating chamber so the vacuum can be maintained if it becomes necessary to stop the pump in the course of dehydration.

A familiar U-shaped mercury gauge is employed to determine if there are any leaks in the vacuum system. Although it is not possible to measure the vacuum accurately, it is sufficient to give the desired results. The Cenco-Hyvac pump is guaranteed to deliver under optimum conditions a vacuum of 0.0003 mm. of mercury, but in the apparatus herein described the guaranteed vacuum can only be approximated. After dehydrating hundreds of pieces of muscle tissue from frozen products, however, it is believed that the system insures adequate dehydration and fixation.

Samples of the frozen product to be dehydrated are cut several millimeters in diameter and about a centimeter in length. If the pump is permitted to function continuously, 24 hours is adequate time for satisfactory dehydration. When the pieces of tissue are thoroughly dehydrated and fixed, they may be removed from the desiccator and immediately thrust into melted paraffin. When placed in the melted paraffin, tissues, if satisfactorily dehydrated, will infiltrate and sink to the bottom of the receptacle within a few minutes. Since the tissues have been subjected to sub-zero temperatures, it is obvious that they will appropriate considerable water from the atmosphere if any appreciable time elapses between removal from the dehydrating chamber and the time of placement in the paraffin. This difficulty can be at least partially overcome by removing from the

freezer the desiccator containing the tissues and permitting them to warm up to room temperature before being quickly transferred to the paraffin.

After the tissues have become infiltrated, they may then be imbedded in paraffin and sectioned. Considerable caution should be exercised if water is to be used to float the sections on slides, since this procedure is likely to cause breaks in the tissue. When examined microscopically, the previous location of the ice formations will appear as spaces in the tissues; and if in handling the sections additional spaces are induced, they may be erroneously interpreted as being ice spaces. The dry method of placing the sections on slides is recommended whenever possible.

HISTOLOGICAL APPEARANCE OF FROZEN POULTRY MUSCLE

The striated muscle tissue used in this study was secured from Plymouth Rock chickens that weighed approximately two and one-half pounds after being drawn. The birds were placed in the various freezing temperatures approximately 24 hours after being killed and were not wrapped or packaged in any way while being frozen. They were held at +3.3°C.(+38°F.) in a cooler for 24 hours previous to freezing. All samples of tissue taken from the frozen birds were cut about three millimeters in diameter and about a centimeter in length. The small pieces of frozen tissue were placed in the dehydrating apparatus which operated at —35.6°C.(—32°F.). After being sufficiently dehydrated and consequently fixed, the tissues were removed from the dehydrating chamber and were infiltrated in paraffin, sectioned at 14 microns, and stained with Delafield's hematoxylin.

All photomicrographs (Figs. 2 to 10) have been magnified 83 times, except Figs. 5 and 7 which were magnified 250 times. The photomicrographs were taken with a Spencer microscope and a Bausch and Lomb type H photomicrographic camera. Except for Figs. 2 and 3, all photomicrographs represent muscle tissue taken from birds frozen in cooled air.

Muscle tissue is composed principally of closely adjoining, parallel, muscle fibers held together by varying amounts of interstitial connective tissue. A number of muscle fibers form bundles of fibers again delimited by connective tissue and designated as primary bundles. A number of these primary bundles compose secondary bundles, etc., until a muscle is formed, many of which constitute the edible tissues of meat and poultry.

It will be noted that the constituent muscle fibers (Fig. 2) closely approximate one another and have a rather uniform and even distribution. The protoplasm of the component fibers, when viewed transversely, presents a homogeneous appearance in that it is not

vacuolated. The fibers contain approximately 75 per cent of water by weight, and the fate of this water during freezing is of particular interest at this time. One factor that affects the size of the ice formations is the rate of freezing, viz., if freezing is rapid, the crystals will be small and if freezing is slow, the crystals will be of a larger order.

If a small piece of chicken muscle (Fig. 3) several millimeters in diameter is frozen in acetone chilled with dry ice to —75.6°C. (—104°F.), freezing will be accomplished in a few seconds. When freezing this rapidly, the water separates from the fiber protoplasm in such a manner that the resultant ice crystals will be extremely small but numerous. The small microscopic vacuoles, as seen in the photomicrograph, are spaces which were previously occupied by ice

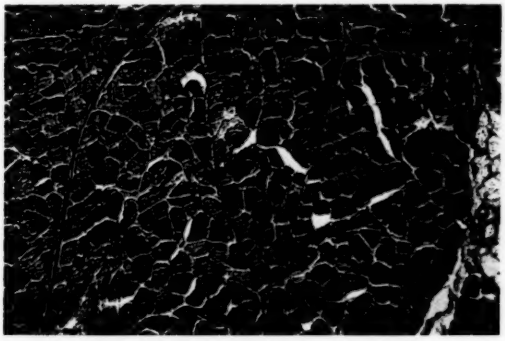

FIG. 2. Never-frozen chicken muscle. X83.

columns but which were removed through dehydration *in vacuo*. It will be noted that the muscle tissue frozen at —104°F. histologically closely resembles the never-frozen tissue.

The tissues of whole birds frozen in air, cooled to approximately —48.4°C.(—55°F.), froze considerably slower than the small piece of muscle immersed in acetone cooled to —104°F. Structurally, the tissue frozen at —55°F. (Fig. 4) shows a marked difference from that shown in the previous figure. The fibers individually considered appear to be quite distorted and are characterized by their jagged outer surfaces. During freezing, water has been displaced to the periphery of the fibers; and when dehydrated, the ice formations disappear leaving the jagged-appearing fibers.

Higher magnification (Fig. 5) reveals that the ice columns appear to have formed within the limits of the sarcolemma. Longitudinal sections show that the ice has formed parallel to the long axis of

the fibers and that the columns of ice appear to extend the length of the fibers. The diameter of the ice columns increases in proportion to temperature increase. When water is frozen within the limits of

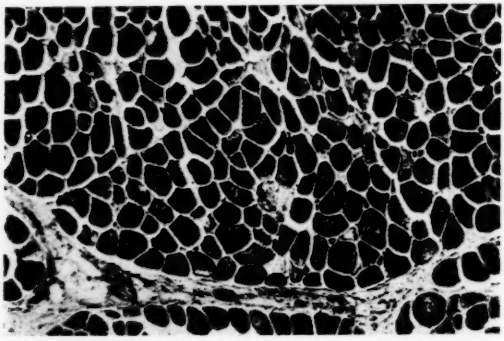

FIG. 3. Small piece of chicken muscle frozen in acetone cooled to —75.6°C. (—104°F.). X83.

the sarcolemma, it may be conveniently spoken of as intra-fiber freezing.

The manner in which water is displaced within the muscle fibers when freezing at the lower temperatures is a curious phenomenon.

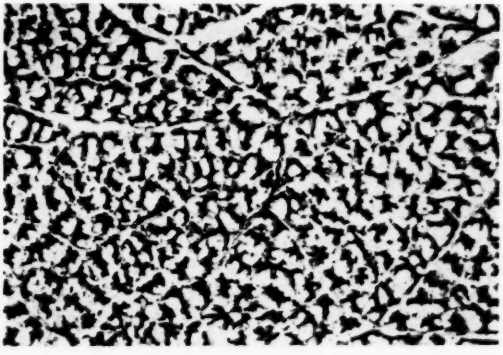

FIG. 4. Chicken muscle frozen at —48.4°C.(—55°F.). X83.

It will be recalled that when a small piece of muscle was frozen at —104°F., the ice formations were evenly distributed throughout the fibers and when the birds were frozen at —55°F., the ice columns assumed a peripheral arrangement. When birds such as those used in this study are frozen in air at about —40°C.(—40°F.), the water

is frozen within the fibers and accumulates approximately in the center of the fibers (Fig. 6) with the fiber protoplasm being distributed at the periphery of the large ice columns. This phenomenon is especially emphasized (Fig. 7) when examined under higher magnification. It will be noted that the sarcolemma appears to have had

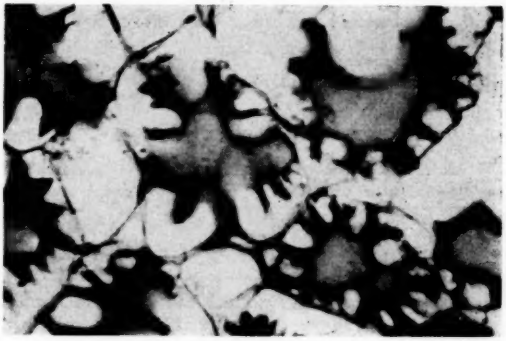

FIG. 5. Chicken muscle frozen at —48.4°C.(—55°F.). X250.

sufficient elasticity to withstand the swelling caused by the large central ice formations.

When birds similar to those used in the previous cases are frozen at —35.6°C.(—32°F.) in air, in some of the fibers intra-fiber freezing occurs (Fig. 8), whereas others lose water which freezes externally

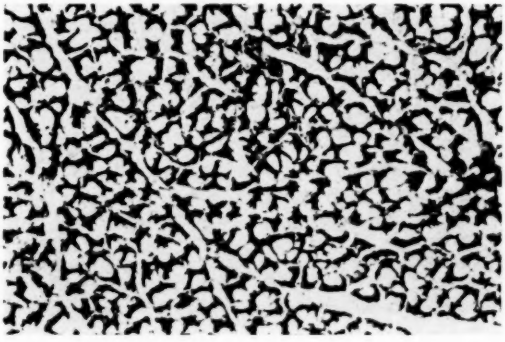

FIG. 6. Chicken muscle frozen at —40°C.(—40°F.). X83.

to the fibers. The spherical spaces indicate the degree of intra-fiber freezing in that such spaces were previously occupied by the large, centrally located ice columns. Upon close inspection of the photomicrograph as evidenced by the nuclei, it will be seen that the fibers have been forced in many instances into compact groups.

If two and one-half pound birds are frozen in air at —26.1°C. (—15°F.) (Fig. 9) extra-fiber freezing occurs exclusively. All the water that freezes at this particular temperature is frozen out of the constituent fibers. It can very clearly be seen in the photomicrograph

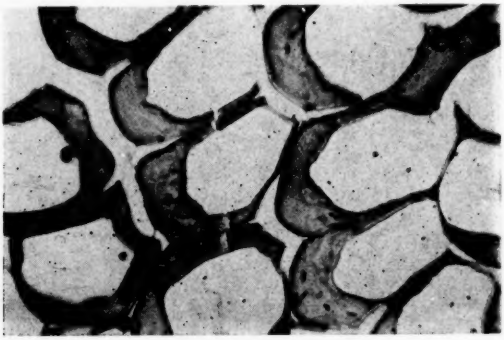

FIG. 7. Chicken muscle frozen at —40°C.(—40°F.). X250.

that the constituent fibers are compressed, many of which appear to be flattened considerably. They have been forced together owing to the pressure created by the external ice formations.

When tissues are frozen at temperatures higher than —15°F., the water is likewise frozen outside of the fibers. Tissues (Fig. 10)

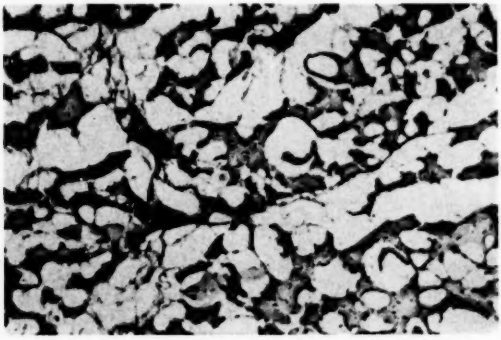

FIG. 8. Chicken muscle frozen at —35.6°C.(—32°F.). X83.

frozen at —13.3°C.(+8°F.) differ from those frozen at —15°F. in that the ice formations become larger and fewer in number; consequently, the component fibers and bundles of fibers are forced into compact-ridge or band-like formations. This phenomenon characterizes muscle tissue that has been slowly frozen.

DISCUSSION

From the foregoing description, it becomes apparent that the rate of freezing is a factor that significantly affects the size, number, and distribution of ice formations. It is appreciated that when birds

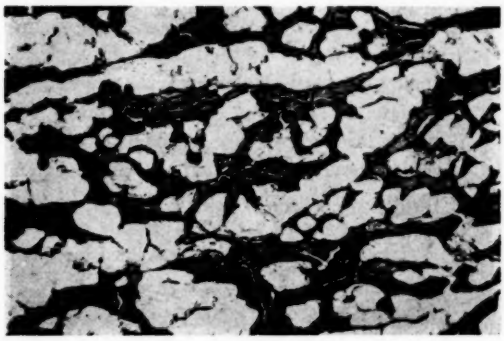

FIG. 9. Chicken muscle frozen at —26.1°C.(—15°F.). X83.

of different size, age, and physiological condition are frozen the histological structure of the frozen tissues may be somewhat different at the various temperatures than herein described. Likewise, precooling conditions and the time of freezing after killing are also factors that may affect the appearance of the frozen tissues.

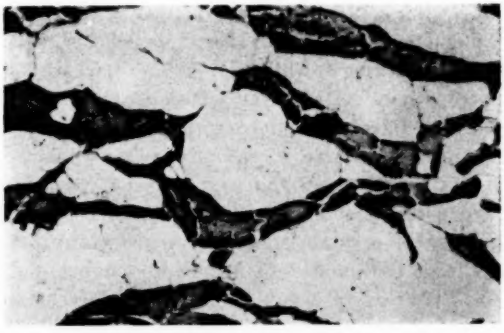

FIG. 10. Chicken muscle frozen at —13.3°C.(+8°F.). X83.

Regardless of freezing method employed, similar striated muscle tissue, if passed through the period of crystallization at the same rate, will exhibit a similar structure so far as ice formations are concerned. If frozen slowly, water will be lost by the fibers and will be frozen externally to the fibers; if frozen rapidly, the water will be

frozen within the fibers. For each method of freezing used, there will be a particular temperature at which the water will be lost by the constituent fibers and such fibers temporarily may be thought of as being at least partially dehydrated.

A particular temperature somewhat lower than the one producing extra-fiber freezing will induce the formation of a single, large column of ice more or less centrally located in the fiber.

Temperatures below those that characterize the single-column formation will cause the formation of several to many intra-fiber ice columns; and if the temperatures are sufficiently depressed, the ice crystals will be evenly distributed throughout the fiber and will be of microscopic dimensions. When freezing occurs within the fiber, the ice columns are arranged parallel to the long axis of the fibers; when freezing is external, the formations are arranged in general parallel to the direction of the bundles of fibers.

If tissues are exposed to temperatures slightly below the freezing point of water, the first crystallization nuclei to appear will be external to the fibers, viz., in the interstitial fluid that bathes the fibers. There are probably several factors responsible for this phenomenon. Perhaps the most important factor is that the lymph has a slightly higher freezing point than the interior of the fiber. While studying the formation of ice in living muscle fibers isolated from frog sartorius muscle, Chambers and Hale (1932) especially emphasized that the sarcolemma acts as a barrier effectively preventing the transmission of the freezing process to the interior of the fibers and that interior freezing in cells may also be delayed by the retardation effects of capillarity owing to the microscopic dimensions of the cells. These two factors may be effective, after death has ensued in the tissues, in delaying freezing in the interior of the fiber.

After the initial formation of the minute external crystallization nuclei, there is a tendency on the part of such crystals to grow in size at the expense of the fiber-contained water. During very slow freezing the external crystals continue to grow until the large ice accumulations force the bundles of fibers into compact-ridge or band-like formations. If freezing is sufficiently rapid, the changes requisite for loss of water by the fibers will not have opportunity to occur and the intra-fiber water will be frozen within the component fibers. The sarcolemma plays an important role in preventing the escape of the fiber-contained water; this is particularly true when freezing at temperatures that are sufficiently depressed to cause internal freezing.

When freezing at the higher temperatures as previously noted, extra-fiber freezing occurs. The manner in which this water is lost by the fibers has been said to be due to a mechanical rupturing of

the sarcolemma which readily permits the escape of water. Nutall and Gardiner (1918), in reviewing Dr. Karl Reuter's 1916 paper, particularly emphasize that the sarcolemma is ruptured by fiber-contained fluid which during the progress of freezing separates from the gelatinous content of the muscle fiber and at a critical point mechanically ruptures the sarcolemma permitting the escape of the fiber-contained water.

It is difficult to ascertain whether all the water lost by the fibers, when freezing at the various slow-freezing temperatures, escapes through a ruptured sarcolemma. Although not confirmed, it appears conceivable that some water may be lost by the fibers during slow freezing through an intact sarcolemma, one that has not previously been ruptured. The sarcolemma may be readily permeable to water when the freezing process is prolonged to the extent that characterizes slow freezing. This permeability factor coupled with the intensified tendency on the part of the external ice crystals to grow at the expense of the fiber-contained water may conceivably explain the loss of at least a part of the water by the fibers and the appearance of the large interstitial ice formations when tissues are frozen at higher temperatures. The pressure exerted upon the fibers by the growing external ice crystals is an additional factor that may promote the loss of intra-fiber water.

The terms fast freezing and slow freezing have been used vaguely and have had no definite meaning. When considering frozen tissues it is the fluid or water contained within the muscle fibers that is of primary interest, since the water external to the fibers is small in comparison with the total amount of water in muscle tissue. The fiber-contained water that freezes at any particular temperature either freezes within the fiber or is lost by the fiber and freezes externally to it. The authors have referred to the former as intra-fiber freezing and the latter as extra-fiber freezing.

SUMMARY

An inexpensive apparatus has been described that adequately dehydrates frozen tissues and simultaneously fixes such tissues by drying. Upon microscopic examination, the previous location of ice formations can be identified as intra-fiber or extra-fiber spaces.

It has been demonstrated that if small pieces of poultry muscle are frozen almost instantaneously, the water freezing within the fibers appears as minute, evenly distributed ice columns. When muscle tissue is frozen somewhat slower, fewer ice columns are present within the fibers, are larger in diameter, and may generally be described as having a peripheral distribution. If the temperature

is further raised, the water will be displaced to the center of the fiber and will appear as a single, large, centrally located ice column. Finally, when the freezing process is sufficiently prolonged, there is a particular temperature at which water will be lost by the fibers and in consequence will freeze externally to the fibers.

Fast freezing has been referred to as intra-fiber freezing and slow freezing has been referred to as extra-fiber freezing, when considering fiber-contained water.

ACKNOWLEDGMENT

The authors are indebted to Mr. W. D. Roth, Swift & Company, for assistance received in obtaining the photomicrographs.

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OCCURRENCE OF CAROTENE IN THE OIL OF *ATTALEA GOMPHOCOCCA MART.* AND ITS RELATION TO VITAMIN A POTENCY

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From time to time reference has been made in the literature to the presence of carotene in palm oils. Gill (1918) has reported its presence in the oils of *Eloeis guineensis (L. Jacq)*, the African palm, and *Eloeis melanococca (Garb)*, the South American species. Rosedale (1935) has recorded a vitamin A potency for the red palm oil of Malaya, *Eloeis guineensis*, of 1,200 yellow units per gram and has further stated that this value has been confirmed by feeding experiments, although the results of the feeding trials are not included in his report. The occurrence in palm oil of carotene (from which the organism can obtain vitamin A through metabolic processes) in amounts almost equivalent to one-half the vitamin A potency of cod liver oil renders the oil of *Eloeis guineensis* a valuable source of the fat-soluble A factor.

The literature available does not furnish data for other species of *Eloeis* nor for palm oils generally, nor have we been able to trace for *Eloeis guineensis* references dealing with variations in carotene content with locality.

We have been interested for some time in nutrition studies connected with native races in the Pacific and Panama, and our initial studies have been concerned more particularly with food values and food-deficiency diseases.

In the course of a preliminary survey of Panama under the auspices of the Gorgas Memorial Laboratory in Panama City, samples of oil prepared from a palm indigenous to the region were secured, and it was deemed desirable to examine them from the point of view of carotene and vitamin A content. These oils are used in the Chagres river area for cooking and cosmetic purposes, and were prepared by the natives of that region.[2]

According to Standley (1928) two genera of oil palm occur in Central and South America, namely *Eloeis* and *Attalea*. *Eloeis mel-*

[1] Commonwealth Fund Service Fellow.

[2] We wish to thank Dr. Herbert C. Clark, Director, and various members of the staff of the Gorgas Memorial Laboratory for obtaining the oils here studied. The samples represent the oils prepared and used by the natives in the village of New San Juan and vicinity, Republic of Panama.

anococca Gaert. is common in the Central American region, is rich in oil, and is known locally as "corocito colorado," "coquito," and "palmiche," the latter two names being common in Costa Rica. Of the *Attaleas*, the *gomphococca Mart.* is one of the largest and most common palms of southern Central America and is known in Panama as "corozo," "corozo gallinazo," and "palm real." Both the oil obtained from the cortex and that yielded by the kernel of the corozo palm nut are used by the natives in rice cooking, and the oil from the cortex is employed as a medicine for colds and cosmetically as a hair dressing.

The samples of oil examined were obtained from the cortex and kernel of the nuts of two varieties of corozo palm, namely "corozo negro" and "corozo gallinazo." We were concerned more particularly with the outer or true palm oils as distinct from palm kernel oil.

EXPERIMENTAL PROCEDURE

The oil constants for the experimental oils, as determined by the official A.O.A.C. methods (1935), are presented (Table 1) with the values for related oils as given by Bolton and Revis (1913) presented for comparison.

TABLE 1

Properties of Experimental and Reference Oils

Oil	Color	Consistency at 20°C. (68°F.)	Saponification No.	Iodine No. (Hanus)	Non-saponification No.
Experimental					
Cortex oil—"corrozo negro".....	Red	Fluid	187.1	87.1	1.54
Cortez oil—"corrozo gallinazo"....	Red-yellow	Fluid	191.0	77.6	0.94
Kernel oil—"corrozo negro".....	Pale yellow	Semi-solid	245.7	15.4	.....
Bolton and Revis (1913)					
Normal palm oil.....	Red	Fluid	199.2	56.3	.....
Normal palm oil.....	Cream	Semi-solid	246.2	14-19	0.12
Cohune oil	Cream white	Semi-solid	254-260	9-12.5	.....

From the table it will be observed that in general the oils of *Attalea gomphococca* are related to those of the true palms but that the oils obtained from the cortex have much higher iodine values.

Examination of Non-Saponifiable Matter for Carotenoids: The intense reddish color of the oils suggested the presence of carotenoids,

and the production of a green coloration upon treatment of the original oil with ferrous chloride confirmed this suggestion. The absence of coloration with concentrated sulfuric, hydrochloric, and nitric acids, when added to an alcoholic solution of the non-saponifiable matter, suggested that the carotenoids present were of the carotene type.

Fifty grams each of the cortex oils dissolved in 200 c.c. of alcohol were saponified with 25 per cent of KOH solution. The soap solutions were evaporated to dryness under a reduced pressure of CO_2, taken up in water, and the water solution of the soaps thoroughly dried and then extracted with successive small quantities of petroleum ether—b.p. 40 to 60°C.(104 to 140°F.). The petroleum-ether solution was golden yellow in color and the color remained on shaking with 90 per cent alcohol, indicating the absence of xanthophylls, rhodoxanthin, and fucoxanthin. The petroleum-ether solution and solutions of the non-saponifiable matter in benzene and carbon-disulfide were subjected to a chromatographic analysis using columns of finely divided chalk and chromatographic alumina. In all cases an examination of the columns failed to show banding, indicating the absence of carotenoids of the xanthophyll type. The carbon-disulfide solution was blood red in color and the benzene and petroleum-ether solutions golden yellow.

A spectroscopic examination of the solutions of the non-saponifiable matter was made with a pocket spectroscope. Two bands were observed in the green and blue in benzene solution and a shift of the bands was observed in carbon-disulfide solution. The approximate readings obtained with the instrument available showed the bands to occur thus: Band 1 at from 530 to 510 mμ. and Band 2, from 490 to 470 mμ. The bands were perfectly plain at the right dilution, no continuous spectrum, such as would have been occasioned by the presence of lycopin in admixture with carotene, being observed.

The spectroscopic observations point to the absence of xanthophylls, rhodoxanthin, and lycopin; this latter carotenoid was further excluded by the absence of a bluish tint in the carbon-disulfide solution of the nonsaponifiable matter persistent with dilution. The material under examination became yellow on great dilution.

Owing to the small quantity of oil available for examination it was not possible to isolate the carotenoids, but the observations recorded above point strongly to the presence of a single lipochrome or closely related lipochromes of the carotene type.

Biological Tests for Vitamin A: Young rats 21 to 28 days old were fed the "depletion diet" specified by the United States Pharmacopoeia (1936) until they ceased to gain weight and had devel-

oped definite eye symptoms indicative of vitamin A deficiency. The animals were then distributed into assay groups in accordance with the requirements of the official U.S.P. assay method. Six animals suitably chosen for weight uniformity and sex distribution were assigned to each group.

The experimental oils were fed at two levels for each oil, and in addition one control and one reference group were run at the same time as the assay groups.

It was found that dosages of 22 mg. for the "corrozo negro" cortex oil and 78 mg. for the "corrozo gallinazo" cortex oil were required daily to restore the animals to health and promote a gain in weight of not less than 12 and not greater than 60 grams over the assay period as required by the U.S.P. method. A dosage of 1.2 mg. daily of the standard pharmacopoeia cod liver oil was fed to the reference group. In the control group four of the six animals had died as a result of vitamin A deficiency before the completion of the assay period.

The data obtained from the assay and the reference groups may be summarized as follows: "Corrozo negro" cortex oil, fed at a daily level of 22 mg., produced an average gain in weight over the test period of 36.8 gm.; "corrozo gallinazo" cortex oil, fed at a daily level of 78 mg., produced an average gain of 40 grams over the assay period; standard reference cod liver oil, fed at the daily level of 1.2 mg., produced an average gain in weight of the reference group, over a similar period, of 38.2 gm. The reference oil contained 3,000 international units of vitamin A per gram. It is obvious from the above figures that the test palm oils do not conform with the United States Pharmacopoeia standard of 600 international units or over per gram of cod liver oil. A simple calculation indicates that the "corrozo negro" cortex oil contains the equivalent of about 158 I. U. of vitamin A per gram, and the "corrozo gallinazo" cortex oil about 48 I. U. per gram.

SUMMARY

By chemical, physical, and feeding experiments it was shown that carotenes occur in the cortex oils of two varieties of the nuts of *Attalea gomphococca*, a palm occurring in Panama.

Vitamin A potencies were determined by feeding experiments and proved to be about 158 I. U. per gram for the cortex oil of the "corrozo negro" variety, and about 48 I. U. per gram for the "corrozo gallinazo" sample.

These vitamin A values, determined biologically, are well below those recorded by Rosedale (1935) for the oil from another variety of palm, *Eloeis guineensis*.

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VARIOUS FATS USED IN DEEP-FAT FRYING OF DOUGH MIXTURES AT HIGH ALTITUDES

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The relative values of different fats used for deep-fat frying of dough mixtures have been compared at the Wyoming Experiment Station.

The study was divided into two parts. The first part consisted of tests to determine the frying life of four different fats when used under household conditions. This included a partial chemical analysis of each fat when fresh and when re-used 6, 12, and 18 hours for frying doughnuts. The flavor, texture, and appearance of doughnuts cooked in the fresh and re-used fats were compared. The effect of storage temperatures upon the development of acid in lard and corn oil was noted as well as factors affecting the absorption of fat in doughnuts.

The second part of the study consisted of formula modifications for doughnuts and the determination of satisfactory frying temperatures at an altitude of 7,200 feet.

EXPERIMENTAL PROCEDURE

The fats used in the above tests were lard, corn oil, cottonseed oil, and a hydrogenated fat. Three lots of lard and two samples of each of the other fats were tested. The above fats, with the exception of corn oil, were secured from the manufacturer with a partial chemical analysis of each lot.

All samples were cared for and stored in a similar manner. They were strained through cheesecloth, after each frying, into tin containers, kept tightly closed, and stored at a temperature of 4.4 to 10°C.(40 to 50°F.). No fresh fat was added during the series of cooking tests. Each lot was used at intermittent periods over several months, which would be comparable with household practice.

Eight pounds was the initial amount of lard and hydrogenated fat for the 12-hour and 16 pounds for the 18-hour series of frying tests. The oils were used in one-gallon lots for the 12-hour and in two-gallon lots for the 18-hour tests. The larger amount of fat would not be used for household frying but for large-quantity cooking.

Three lots of lard which are commonly found on the market were tested, namely, Grade I, smooth leaf lard; Grade II, smooth leaf lard; and a leaf lard of grainy consistency.

TABLE 1

Chemical Changes in Fats Upon Re-Use

Fat	Sample No.	Iodine No. (Wijs)				Free fatty acids (as oleic)				Smoking point (°F.)			
		Unused	Re-used			Unused	Re-used			Unused	Re-used		
			6 hr.	12 hr.	18 hr.		6 hr.	12 hr.	18 hr.		6 hr.	12 hr.	18 hr.
						pct.	*pct.*	*pct.*	*pct.*				
Grade I leaf lard	1	65.0	64.2	61.9	.....	.277	.350	.510	.....	345	335	330	.....
Grade II leaf lard	2	64.9	60.7	55.9	53.0	.367	.475	.600	.755	348	338	328	315
Leaf lard, grainy	3	68.2	64.2	54.5	.....	.335	.505	.677	.....	340	332	328	.....
Hydrogenated vegetable fat	1	60.2	58.5	56.4	.....	.031	.051	.258	.....	430	390	360	.....
Hydrogenated vegetable fat	2	62.9	59.1	57.0	55.4	.039	.105	.292	.522	428	400	350	338
Corn oil	1	117.6	114.7	111.7	.....	.055	.142	.295	.....	440	390	370	.....
Corn oil	2	129.6	127.3	126.0	121.9	.082	.063	.181	.299	445	420	380	365
Cottonseed oil	1	100.8	97.8	93.7	.....	.030	.177	.229	.....	440	.....	390	.....
Cottonseed oil	2	110.2	107.5	101.8	99.4	.026	.058	.148	.222	440	405	390	380

DISCUSSION

The data show that the free fatty acids of unused lard from the above lots varied from .277 per cent for Grade I to .367 per cent for Grade II (Table 1). There were also slight variations in the acid content of different samples of unused vegetable oils and hydrogenated fat. It is evident that free fatty acids develop in all of the above fats upon re-use.

The acidity increase with 12 hours' frying, however, was not sufficient to cause disagreeable flavors in the doughnuts. With continued use for 18 hours both lard and hydrogenated vegetable fat developed slightly more free fatty acids than vegetable oils. The flavor of these doughnuts was inferior to those cooked in the fresher fat.

The iodine number of all fats decreased upon re-use. The decrease was greatest in the grainy lard, amounting to 13.7 units as compared with 4.1 units for the smooth lard after the 12-hour test period.

It can be noted that the smoking point was lowered considerably in the re-used fats (Table 1). The decrease was less for lard than for the other fats during similar periods of frying. These data indicate that deterioration has been progressive with continued use of the fats.

Chemical analyses of samples of lard are given (Table 2). Iodine numbers check within one unit in duplicate tests from the same lot. Variations in free fatty acids are small.

A long frying life is desirable from an economical standpoint, which may be prolonged by proper care and storage of the fat. Storing lard in a cool place and keeping it tightly covered is of great importance in preventing rancidity of fresh as well as re-used lard. Unused lard stored at 21.1 to 23.9°C.(70 to 75°F.) for six months developed .045 per cent more acid than lard from the same lot stored at 10 to 15.6°C.(50 to 60°F.) for this period of time. There was little difference in the acidity of unused corn oil stored at these different temperatures for six months. The above fats were kept in tin containers tightly covered.

Home-rendered lard, which became rancid a few months after it was made, had an acid content of 1.77 per cent. This fat, with a high acidity, imparted unpleasant flavors to the food. Attempts to recondition this rancid lard proved unsuccessful.

The percentage of free acid in a used fat has been considered a fairly reliable measure of the extent of its breaking down and imparting poor flavors to foods. Porter, Michaelis, and Shay (1932) reported an objectionable flavor in doughnuts with two per cent acid in the frying medium. This was considerably higher than the acid de-

TABLE 2

Duplicate Determinations for Iodine Number and Free Fatty Acids in Three Samples of Lard

Sample and grade	Test	Iodine No. (Wijs)				Free fatty acids (as oleic)			
		Unused	Re-used			Unused	Re-used		
			6 hr.	12 hr.	18 hr.		6 hr.	12 hr.	18 hr.
A	1.....	65.1	64.3	62.4	....	.275	.350	.513	.....
Grade I leaf	2.....	64.9	64.1	61.5	....	.278	.350	.508	.....
lard, smooth	Av.....	65.0	64.2	61.9	....	.277	.350	.510	.....
B	1.....	64.7	60.6	55.6	53	.370	.480	.605	.740
Grade II leaf	2.....	65.1	60.8	55.8	53	.365	.470	.595	.770
lard, smooth	Av.....	64.9	60.7	55.7	53	.367	.475	.600	.755
C	1.....	68.0	64.6	55.0	....	.336	.500	.675	.....
Leaf lard,	2.....	68.5	63.9	54.1	....	.335	.510	.680	.....
grainy	Av.....	68.2	64.2	54.5	....	.335	.505	.677	.....

veloped in the fats in these experiments in which the highest amount, .75 per cent, was found in re-used lard.

The discoloration in lard and hydrogenated fat apparently had little effect upon the flavor of foods cooked therein. In the case of lard the distinctive lard flavor was less pronounced in doughnuts after this fat had been re-used several times. The reason for this has not been determined but was evident in each of the lards tested.

The smoking points of unused fats at a high altitude of 7,200 feet were approximately the same as at sea level. The method recommended by the American Oil Chemists Society (1933), in which a smoke box was utilized, proved the most satisfactory. The thin atmosphere made it very difficult to see the smoke by the method of Blunt and Feeney (1915).

Whether the above fats after 12 to 18 hours of use had deteriorated to the extent that the products cooked therein would be unsafe for human consumption is a matter for further investigation. The judges suffered no apparent ill effects as a result of eating the doughnuts.

It is evident that under the same conditions of frying, doughnuts absorbed approximately the same amount of each of the different fats (Table 3). The fat was extracted with ether on duplicate samples. However, the absorption varied with many other factors, such as formula changes, temperature for cooking, handling of the dough, and the size and shape of the doughnuts. In a formula with 50 per cent soft-wheat and 50 per cent hard-wheat flour, the fat extracted from the doughnuts averaged 30 per cent, or about one tablespoon to a doughnut. With 75 per cent hard-wheat flour in the mix there was 21 per cent fat in the doughnut.

Doughnuts cooked at a temperature of 196.1 to 198.9°C.(385 to 390°F.) absorbed two per cent more fat than those cooked at 176.7 to 179.4°C.(350 to 355°F.) with both corn oil and hydrogenated fats as frying mediums. This was no doubt due to a rougher surface, the crust forming so rapidly at the high temperature that the leavening burst the shell, resulting in an uneven surface. From the results of other investigations by Woodruff and Blunt (1919), it would appear, other things being equal, that cooking at lower temperatures usually means more absorption of fat.

Raised doughnuts made from a comparatively rich bread dough with Fleischmann's yeast contained 20.8 per cent of fat, which is approximately the same as that extracted from less rich cake doughnuts. Chocolate doughnuts with the addition of nuts and mashed potatoes contained 28.6 per cent of fat.

TABLE 3

Absorption of Fat in Doughnuts and Factors Affecting It

Type	Handling dough	Variation in flour	Frying medium	Temperature for cooking	Time cooked	Fat [1]	Remarks
				°F.	min.	pct.	
Cake	First roll	75% hard-wheat, 25% cake flour	Wesson	350-355	3	21.3	Absorption was approximately the same with different frying mediums.
Cake	First roll	75% hard-wheat, 25% cake flour	Lard	350-355	3	21.1	
Cake	First roll	75% hard-wheat, 25% cake flour	Crisco	350-355	3	21.6	
Cake	First roll	75% hard-wheat, 25% cake flour	Corn oil	350-355	3	20.1	
Cake	First roll	75% hard-wheat, 25% cake flour	Corn oil	350-355	3	22.5	More handling of dough lessened absorption.
	Re-roll	75% hard-wheat, 25% cake flour	Corn oil	350-355	3	19.1	
	Second re-roll	75% hard-wheat, 25% cake flour	Corn oil	350-355	3	18.6	
Cake	First roll	50% hard-wheat, 50% cake flour	Corn oil	350-355	3	30.7	Use of soft-wheat flour increased absorption.
Cake	First roll	50% hard-wheat, 50% cake flour	Lard	350-355	3	30.0	Use of soft-wheat flour increased absorption.
Chocolate cake	First roll	50% hard-wheat, 50% cake flour	Corn oil	350-355	3	28.6	Mashed potato, nuts, and chocolate were added to this formula.
Cake	First roll	50% hard-wheat, 50% cake flour	Corn oil	350-355 385-390	3	26.8 28.6	Outer surface rougher at higher temperatures.
Commercial cake	Dropped from machine	.....	.....	.....	....	21.6 [2] 21.6 22.4	Samples were approximately the same weight.
Commercial cake	Pastry tube	.....	.....	.....	....	17.2 [2] 20.4	Weight of No. 1—35.4; weight of No. 2—47.8 gms.
Raised	Bread dough	Hard-wheat flour	Crisco	350-355	3	20.8	Absorption varied slightly from cake doughnuts.

[1] Average of two extractions. [2] Extractions of separate doughnuts.

Commercial doughnuts dropped from a doughnut machine varied only slightly, one from another, in fat content. The average was 21.8 per cent. A second lot dropped from a pastry tube varied considerably in size and shape and likewise in fat content. A small one contained 17.2 per cent of fat, whereas the second, thicker and larger, contained 20.4 per cent (Table 3).

Just how much fat a doughnut should carry to make it desirable from the standpoint of palatability is questionable. A doughnut must carry a certain percentage of fat to be a real doughnut. Doughnuts containing 25 to 30 per cent fat scored higher in eating qualities than those containing 18 to 20 per cent. An extremely low fat absorption can therefore not be used as a criterion for palatability. From the standpoint of ease of digestion a low absorption is considered best.

The second part of the study consisted of determinations of the best frying temperatures at high altitudes as well as formula modifications for rich dough mixtures.

The temperatures tested for frying ranged from 176.7 to 204.4°C. (350 to 400°F.). Doughnuts fried at 176.7°C.(350°F.) for three minutes were a golden brown color; those fried at 185°C.(365°F.) for two minutes were a deep brown color; and those fried at 198.9°C. (390°F.) for one and one-half minutes were very dark brown. The deepening in the color was more pronounced for re-used than for fresh fat at the above temperatures. This range of temperatures was tested with corn oil and also hydrogenated fat and refers to the actual frying temperatures.

It was difficult to evaluate the various data on the best cooking temperatures at low altitudes and to establish a satisfactory norm for comparison, since temperatures ranging from 350 to 400°F. were listed as desirable for frying doughnuts at sea level. The upper limit was not satisfactory at an altitude of 7,200 feet. It seems plausible that such should be the case, since the volatile constituents are lost more rapidly at the higher elevations. Since moisture fries out of the food more speedily, the intrinsic heat would rise more rapidly in the food to the end that at the same temperature, other things being equal, a darker brown product would result.

VARIATIONS IN FORMULAS

A survey of low-altitude doughnut formulas revealed almost as many as for cakes. The recipes must be balanced as for cakes, and when balanced, proportions vary. In an analysis of 25 low-altitude, household formulas counting flour 100 per cent, there was the following range in proportions for plain cake doughnuts: sugar 17 to 59

[1] Average of two extractions. [2] Extractions of separate doughnuts.

per cent, eggs (whole) 10 to 28 per cent, milk 36 to 55 per cent, fat 2 to 12 per cent, baking powder 2 to 7 per cent.

Most of the doughnuts made by sea-level formulas at the high altitude of 7,200 feet were very poor in quality. The doughnuts cracked and had a high fat absorption which is usually an indication of a too-rich dough. Frequently they were hard and brown. The following proportions gave a fairly satisfactory product, counting flour 100 per cent, of which one-half was hard-wheat and one-half soft-wheat flour: 40 to 43 per cent sugar, 16 to 20 per cent whole eggs, 43 to 53 per cent milk, 2 per cent fat, and 1 to 1.8 per cent baking powder.

It can readily be noted that the greatest variation between low-altitude and high-altitude formulas occurred in the leavening, which was less than the minimum at sea level. This can easily be understood, since at higher levels the atmospheric pressure is lower, hence the internal pressure in the dough must be decreased by using less baking powder or soda. The shortening also was at the lower limits, since too much tended to weaken the structure and open it up.

Sugar made the doughnuts more tender and gave a good flavor. The optimum amount that could be used depended upon the strength of the flour. It was possible to use larger proportions with hard-wheat flour in the mix.

The type of flour undoubtedly influenced the eating quality to a a great extent. Cake flour, which may be used 100 per cent at low altitudes, did not prove satisfactory in this proportion in the high-altitude formulas. The doughnuts cracked and soaked up grease. The proportion of 50 per cent cake flour and 50 per cent hard-wheat flour gave a tender product with a normal absorption of fat and less tendency to crack. Hard-wheat flour used 100 per cent toughened the product.

Either whole eggs or egg yolks could be used. When egg yolks were used in excess, however, the doughnuts became too short and cracked open, probably because of the high fat content and lack of albumen in the yolks. An excessive amount of whole eggs produced a tough doughnut, possibly due to the binding effect of the albumen. The proportion of one-third yolks and two-thirds whole eggs gave good results.

It was also noted that keeping the mix on the soft side helped to prevent cracking, hence the liquid in the high-altitude formulas was higher than the average at sea level.

It is evident that many of the modifications outlined above follow the same trend as successful modifications for butter cakes at high altitudes.

CONCLUSIONS

From these experimental tests it may be concluded that:

1. Doughnuts of a good quality can be secured with each of the frying mediums tested. Judges could rarely distinguish the fats used with the exception of lard. Only the cheaper grade of lard was rated undesirable.

2. Fats used in frying showed consistent decreases in the iodine number and smoking point but increases in acidity.

3. The smoking points of unused fats were approximately the same at low and high altitudes.

4. The reheating and re-use of a fat up to 12 hours did not seem to affect the quality or flavor of the product cooked therein.

5. Fat absorption is not dependent upon the kind of frying fat, since the absorption of fat in doughnuts was found to be approximately the same for four different fats, using the same formula and frying temperature.

6. The amount of fat absorbed varied with formula changes, the temperature used in cooking, and the amount of mixing.

7. The high temperatures for frying frequently recommended at low altitudes were not satisfactory at an altitude of 7,200 feet. Lowering the frying temperature prevented a too-deep coloring while the food was cooking.

8. Formula modifications were necessary for rich cake doughnuts at an altitude of 7,200 feet to prevent the cracking of doughnuts and a high fat absorption while cooking.

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VITAMIN CONTENT OF CERTAIN PACIFIC FISH OILS

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The value of fish body and liver oils for medicinal use lies largely in their vitamin A and D content. Since it has been abundantly demonstrated that these products vary greatly in such content, new data concerning commercial or laboratory specimens of known origin appears worth recording.[1]

It has been possible to examine in this laboratory offal, body, and liver oils of important varieties of Pacific coast fish over a period of three years.[2] Commercial oils have been studied as well as the oils obtained in the laboratory from the livers and other parts of freshly caught fish. All of the data reported were obtained by the official U.S.P. XI method for the biological determination of vitamins A and D and by the use of the Lovibond tintometer and antimony trichloride reaction for the colorimetric estimation of vitamin A.

A total of 65 oils was examined, 16 of which were extracted in the laboratory from livers or offal. All of the oils were kept at about —4°C.(24.8°F.) in the dark, and, although some of the assays were repeated one to two years after the oils were prepared or received, no deterioration as to either vitamin A or vitamin D could be detected.

EFFECT OF SEPARATION PROCESS ON SARDINE OILS

1934 Samples: The samples of sardine oil numbered 13 to 20[3] (Table 1) were prepared by the plant on board a fish reduction ship off the central California coast. The fish were caught and reduction carried out late in August or early in September, 1934, at the beginning of the fishing season. The oils were produced for experimental purposes and do not represent commercial grades. Two processes of separation, designated A and B, were used and the separation was carried out at varying periods after the fish were caught.

Another series of eight samples of commercially prepared sardine oils were received in July, 1934. These were prepared by two large California sardine-reducing plants and were reported to have been

[1] Assistance in this study was rendered by Works Progress Administration Official Project 665-08-3-30, Unit A-24.

[2] Many of the samples both of fresh livers and of commercial oils were procured by the National Oil Products Company of New York and San Francisco. We acknowledge with pleasure the generous coöperation of this company.

[3] These samples were furnished by Stanley Hilyer, Monterey, California.

TABLE 1

Vitamins D and A in Sardine Oils

Oil No.	Season	Description	Vitamin D	Lovibond blue	Vitamin A[1] estimated from L.B.U.
			U.S.P. units per gm.	*Units per gm.*	*U.S.P. units per gm.*
13	September,	Experimental oils, separated by Proc-	37	.....	.....
16	1934	ess A, 27 and 29.5 hours after catch.	37	.....	.....
17	September,	Process A, 4 and 6.5 hours after	100	.....	.....
20	1934	catch.	More than 37	.....	.....
14	September, 1934	Experimental oil, separated by Process B, 27.5 hours after catch.	74	.....	.....
18	September, 1934	Process B, 4.5 hours after catch.	More than 37	.....	.....
1A			18	30	.....
1B			18	40	.....
2A	September,	Commercial oils prepared by Process	Less than 37	30	.....
2B	1934	C, boiling and settling separation.	Less than 37	50	.....
3A			Less than 37	30	.....
1C			48	67	.....
2C	September,	Experimental oils prepared by centri-	More than 37	67	.....
4A	1934	fuge separation, Process A.	56	13	.....
78A	January, 1938	Oil from bodies of sardines, extracted by steam in laboratory.	95	56	.....
79A	January, 1938	Oil from heads, tails, and viscera of same sardines, extracted by steam in laboratory.	70	120	600
79B	January, 1938	Oil from breaker tank, spot sample.	35-50	100	500
6079	January, 1938	Oil from breaker tank, composite sample.	70-80	150	750
80A	January, 1938	Oil from first settling tank, spot sample.	95	115	575
6080	January, 1938	Oil from first settling tank, composite sample.	95	120	600
81A	January, 1938	Oil from centrifuge, spot sample.	95	130	650
81B	January, 1938	Oil from centrifuge after washing, spot sample.	95	138	690
6087	January, 1938	Refined oil, composite sample representing 607 tons raw fish, 33 hours' run.	95	90	450[2]
495	January, 1938	Commercial sample, 1938 season.	95	.....	.....

[1] This calculation is probably indicative of the vitamin A value only when the number of Lovibond blue units is more than 100 per gram. [2] Biological test showed this oil to contain 300 U.S.P. units per gram.

separated by direct centrifugation in some cases and by boiling and settling in others. The reduction processes used are described as follows:

Process A: The fish were drained of sea water and held in bins, then fed to a continuous-process steam cooker. The cooked fish were fed to a press in which the meal and liquid portions were separated. The liquid portion was separated from entrained solids and the liquid then treated in a Sharples supercentrifuge to separate the oil from the watery portion. The process was designed to complete the operation in minimum time and with minimum use of heat and exposure of the mixture to air.

Process B: The same treatment described under Process A was used, but the oils were recovered without the formation of emulsions. There is a certain amount of emulsification of the oil in the adjacent layer of "stick water" in the usual separation, this emulsion later being broken and the oil therein added to the rest of the unemulsified oil. In Process B such recovered emulsified oil was not mixed with the samples submitted, that is, Samples 14 and 18. Samples 13 and 17 represent whole fish oil, emulsified as well as non-emulsified.

Process C: This is the method most commonly used formerly in fish-reduction plants. The fish were macerated, cooked in a steam cooker, and the mixed oil and "stick water" run off into three or more connected settling tanks. The oil was drained off after the mixture was cooked and separated completely by gravity.

Sardine oil samples No. 13 and 17 are directly comparable since they were separated by the same process, A, the only difference being that the fish were held for 27 hours before the separation of oil was made in the case of Sample 13, and for only four hours in the case of Sample 17. It is difficult to judge whether this difference in time during which the body oil remained under the influence of naturally present enzymes and microörganisms is sufficient to account for the definite inferiority in antirachitic value of Sample 13 (Table 1). Samples 14 and 18, separated by Process B, are similarly comparable but the maximum value for Sample 18 was not determined. It is interesting to note that Sample 14 produced by Process B is of higher antirachitic value than Sample 13, made from the same fish by Process A. Sample 16 made by Process A was not tested for its maximum value but was probably of the same potency as Sample 13. Sample 20 also made by Process A was apparently similar to Sample 17.

It may be concluded that oil emulsified in the separation process is likely to have suffered some loss of antirachitic activity, (13 *versus* 14) and that the lapse of 23 hours (13 *versus* 17) before separation of oil from the fish bodies decreases the vitamin value of the oil.

Only two of these oils obtained in 1934 contained as much as 74 units of vitamin D per gram and only three of the remaining number contained as much as 37 units per gram (Table 1). Apparently the centrifuge method of separation used for 1C and 2C was effective in conserving a larger part of the vitamin than that retained by the four oils, 1A, 1B, 2A, 2B, separated by the longer process, C. The former samples, 1C and 2C as well as 4A, were obviously similar in antirachitic value to the centrifuged samples in the Hilyer series. Only one of the 1934 sardine oils met the standard set by the U. S. Pharmacopoeia XI for cod liver oils.

Vitamin A was determined by the antimony trichloride reaction of Carr and Price (1926) and quantitative estimation attempted by comparison of the depth of blue color in a Lovibond tintometer with that obtained using the U.S.P. reference cod liver oil and the procedure prescribed in the British Pharmacopoeia (1932). Faint blue colors were obtained with all the sardine oils but none of them was deep enough to justify quotation. It was concluded that vitamin A is present in negligible amounts only in all these sardine oils. However, Samples 1C and 2C, separated by centrifuging, had definitely larger blue values than the other samples. Whatever vitamin A is present is conserved by the lower temperature and less aeration involved in the centrifuging separation.

1938 Samples: Another set of samples of sardine oil was secured in the latter part of the fishing season in January, 1938.[4] Spot samples were taken at the reduction plant on January 28. During the continuous 33-hour run following, while 607 tons of fish were processed, other samples were taken at 15-minute intervals, composited, and small amounts forwarded to us. Both spot and composite samples were taken so as to represent the product of various stages of the reduction process.

The process used in this case was essentially a combination of those described previously as Processes A and C. The fish were cooked with steam in continuous cookers for about 20 minutes, the liquid pressed out by continuous presses, and run into a breaking tank in which the oil was allowed to separate from the "stick water" by gravity (Samples 79b and 6079). The first oil tank (Samples 80a and 6080) then received the overflow of oil from the breaking tank, which was fed as fast as the machine could take it into the DeLaval oil purifier centrifuge. Some drainer oil, obtained as overflow from the bodies of the fish canned by a boiling process, was allowed to enter the oil flow just before the stream entered the centrifuge.

[4] These samples were obtained January 28-31, 1938, at the Pittsburg, California, plant of the F. E. Booth Company through the coöperation of Mr. T. D. Sanford.

The oil from the centrifuge (Samples 81a and 6081) was washed with water and centrifugalized twice to produce the refined oil (Samples 6087 and 81b). Stearin is removed from this oil later by cold-press filtration but no samples of the final product were tested.

Samples of the raw fish were collected at the same time with the spot samples, and oil from the bodies and the offal was obtained by steam distillation in the laboratory (Samples 78a and 79a). All the samples taken from the settling tank, centrifuge, and purifiers were found to have vitamin D content at least equal to that of the reference cod liver oil, 95 units per gram. The samples taken from the breaker tank were less potent, 35 to 80 units per gram. This is difficult to explain and may be fortuitous. The laboratory extracted body oil was found more potent than the offal oil, again perhaps a matter of chance.

It is interesting to observe the wide difference in antirachitic potency of the sardine oils obtained in these two different years and at opposite ends of the fishing season. Lovibond tests made on all these sardine oils showed them to be as much superior in vitamin A content as in vitamin D to the 1934 samples. There appeared to be three to four times as much vitamin A as in the 1934 samples although still somewhat less than the quantity needed to allow direct comparison with the reference oil. Only one oil, Sample 6087, the purified final product, was tested biologically. This was found to contain 300 U.S.P. units per gram as compared with 450 estimated from the blue unitage.

Apparently the content of both vitamins A and D was increased in about the same ratios in the second series of sardine oils, as compared with the first series, a condition possibly due to greater average age and size of the 1938 catch than that of 1934.

The vitamin potency and acceptable taste of these relatively cheap sardine oils cause the writers to wonder why they are not available for children as well as for poultry. Migrant and relief families are supplied high-priced cod liver oils which are no more acceptable nor effective than these sardine oils, now sold at low prices to poultry producers on vitamin specifications.

Bills, Massengale, Imboden, and Hall (1937) reported one large lot of California sardine oil made from the whole fish as having 110 units of vitamin D and 1,200 units of vitamin A per gram. Differences, both of season and year as well as method of reduction, may account for the variation in potencies reported for sardine oils.

VITAMIN CONTENT OF SALMON OILS

Seven commercially produced salmon offal oils, made from cannery trimmings, and two salmon liver oils, extracted in the labora-

TABLE 2

Vitamins D and A in Salmon Oils

Oil No.	Description	Vitamin D	Vitamin A	Lovibond blue	Vitamin A estimated from L.B.U.
		U.S.P. units per gm.	*U.S.P. units per gm.*	*Units per gm.*	*U.S.P. units per gm.*
6F	Oil from pink salmon offal, cooked 20 min. at 100°C.(212°F.), separated mechanically.	142	.....	33	.....
6C	Oil from mixed pink (about three-fourths), red, and dog salmon offal cooked at 115.6°C.(240°F.) for 25 min., separated by centrifuging.	123	.....	150	885
6D	Oil from red salmon offal, steam-cooked 20 min. at 100°C.(212°F.), recovered by mechanical separation.	114	.....	167	985
6E	Oil from red salmon offal, steam-cooked 20 min. at 100°C.(212°F.), recovered by centrifuging.	58	.....	0	.....
6G	Oil from red salmon offal, cooked at 115.6°C.(240°F.) for 25 min., separated by gravity from press liquors.	95	.....	100	590
6A	Edible or medicinal salmon oil (contains no liver oil) prepared by F3 Packing Co., by steam-cooking and centrifuging salmon body products.	Less than 95	525	217	1,280
6B	Another sample similar to 6A. Both 6A and 6B were bright red in color.	95	44	30	.....
6H	Oil extracted in laboratory with petroleum ether from dog salmon livers received Nov. 15, 1934.	475	5,800	2,900	17,000
6I	Oil extracted in laboratory with petroleum ether from silver salmon livers received Nov. 15, 1934.	570	57,600	6,700	39,500
	Reference cod liver oil	95	3,000	510	3,000

tory, were tested for their vitamin A and D content. Two of the offal oils were labelled as edible, medicinal, canning oils prepared by a northwestern packing firm. The other five were commercial samples prepared as described (Table 2). All of the offal oils were from salmon caught in lower Alaskan waters. Five of these offal oils contained 95 to 142 units of vitamin D and were therefore within the range of standard cod liver oil. The other two were found to be below this standard. Interestingly enough, 6A and 6B, labelled me-

dicinal or edible, were among the less potent of the samples. It may be significant that the oils obtained wholly or chiefly from pink salmon offal were the most potent of all, 123 and 142 units per gram.

Harrison, Anderson, Holmes, and Pigott (1937) report also that pink and chum salmon wastes were better sources of vitamin D than the Chinook salmon. They found that sockeye and silver salmon oils resembled the pink and chum in their vitamin D content, about 300 units per gram. None of the offal oils examined in our laboratory had antirachitic values of this order nor of the order found by Tolle

TABLE 3

Water and Fat Content of Fish Products

Raw material used	Date received	Oil No.	Water	Fat
			pct.	*pct.*
Dog (keta) salmon liver	Nov. 16, 1934	6H	76.5	1.8
Silver (coho) salmon liver	Nov. 16, 1934	6I	77.0	2.6
Sea bass *(Cynoscion Macdonaldi)* liver				
I	Dec. 6, 1934	12A	55.8	22.5
II	Jan. 14, 1935	12C	52.3	23.8
III	Jan. 26, 1935	12E	59.3	20.9
IV	Mar. 25, 1935	12ge	56.6	27.2
California mackerel liver				
I	May 31, 1935	10E	74.0	4.0
II	Sept. 21, 1934	10B	42.4	23.2
California bluefin tuna *(Thunnus saliens)* liver				
I	Oct, 1, 1934	5C	73.0	8.6
II	Apr. 1, 1935	5E	72.4	5.0
Striped tuna liver	May 9, 1935	5G	61.2	13.0
Tuna stomachs and contents	Oct. 1, 1934	5D	74.5	3.7
Basking shark *(Cetorhinus maximus)* liver	Jan. 30, 1935	8D	13.6	75.8

and Nelson (1931) in similar offal oils. Bailey (1936) reported 50 and 67 units of vitamin D and very little vitamin A per gram in the oil of canned British Columbia sockeye and pink salmon.

The livers from which Oils 6H and 6I were extracted were received in frozen condition at the laboratory, immediately ground, partly dried, and extracted with petroleum ether. These liver samples contained a smaller oil content than was shown by any of the other livers studied (Table 3). The oils were dark, viscous, and rich in sterols. The two appeared to be of quite similar vitamin D value, close to 500 units per gram. Harrison, *et al.* (1937) reported 150 to 400 units per gram for three Chinook liver oils.

The vitamin A content of the salmon oils was determined chiefly by the colorimetric method although the biological method was used in four cases also in order to check the accuracy of the "blue-unit" method. None of the salmon offal oils could be said to be of notable value as a source of vitamin A, the best being 6A with 525 units per gram by biological test and 1,280 by tintometer. Since in this, as in every case, the biological evidence must be considered superior, the best offal oil can be said to contain less than the minimum vitamin A content of approved cod liver oil. Four of the other offal oils had somewhat similar but lower vitamin A content and two of them practically none. These values are similar to those reported by Truesdail and Boynton (1931) for salmon body oils but are much lower than those of Tolle and Nelson (1931).

The salmon liver oils proved to be rich in vitamin A, that of the silver salmon being equal to some of the tuna or sea bass liver oils, 57,600 units per gram, as shown by the biological method. The dog salmon liver oil had 17,000 units per gram by the antimony trichloride test, but only 5,800 by the biological method. Harrison, *et al.* (1937) reported 4,000 to 8,000 units of vitamin A for Chinook liver oil.

The lower vitamin A content of the dog salmon liver oil confirms the statements made by Tolle and Nelson (1931) that the deeper colored salmon (body) oils are richer in this vitamin. This did not hold among the offal oils examined in the present series, however, since the deep red samples, 6B and 6E, were the poorest in vitamin A as well as in vitamin D. Possibly these samples were made from offal containing less viscera and more heads, backbones, and collars of Chinook and other red salmon than the offal examined by Tolle and Nelson. Harrison, *et al.* (1937) found a good deal of variation in the vitamin A of various kinds of Chinook offal.

VITAMIN CONTENT OF TOTOABA LIVER OILS

Four shipments of totoaba (white sea bass) livers from fish caught in the Gulf of Lower California were received at the laboratory in the autumn and early spring of 1934-35, each lot, shipped frozen, consisting of eight or ten livers. In each case the extraction of these livers was carried out at once. In some cases the livers were minced, partly dried at low temperature over the water bath, and the oil then removed by refluxing with ethyl or petroleum ether. Some of the livers were steam distilled and the oil separated by gravity and some were extracted directly with ether. The first-mentioned method was found to be speediest and most efficient and was used to obtain most of the oil for the vitamin tests. In most cases these livers were grey-

ish in color owing to their high fat content, 20 to 25 per cent of the fresh weight (Table 3). In one case the ether extract of partially dried livers was compared as to vitamin A with that obtained after complete drying. The oil from the completely dried livers was found by biological test to have lost about one-third of its original vitamin A content. However, such loss through oxidation did not occur in the case of the antirachitic vitamin.

The vitamin D content of these oils varied from 1,400 to 3,500 units per gram, the latter in oil from the livers of lowest fat content. Bills, *et al.* (1937) reported on two samples of liver oils of fish of this or a closely related species as having 6,000 and 1,200 units of vitamin D per gram.

The vitamin A content of these oils was astonishingly large, ranging from 180,000 units per gram in the supernatant liquid oil of livers No. II down to 46,000 in the ether extract of a completely dried portion of livers No. IV. Bills, *et al.* (1937) reported in similar liver oils 55,000 and 56,000 units of vitamin A per gram.

One sample of medicinal halibut liver oil, 14d,[5] was assayed along with the totoaba and tuna liver oils for comparison and found similar to them in vitamin A but inferior in vitamin D potency.

VITAMIN CONTENT OF TUNA OILS

Two lots of fresh bluefin tuna livers shipped frozen from fish caught in lower California waters and one lot of striped tuna livers shipped frozen from Honolulu, taken from fish caught in Hawaiian waters, were extracted with ether in the laboratory in the usual fashion. One experimental and two commercial tuna offal oils were also examined but none of these was of notable vitamin value. The vitamin A and D values of all the tuna liver oils, however, were of the high order reported in previous studies.

Non-saponifiable extracts made with every precaution to prevent aeration from two of the tuna liver oils and from one of the sea bass liver oils were found to have lost a considerable amount of the vitamin A potency of the original oils as judged by the relative vitamin D content of oils and extracts.

VITAMIN CONTENT OF MACKEREL OILS

Two large shipments of fresh mackerel livers were received frozen from southern California in September, 1934, and May, 1936.[6] The

[5] This oil was furnished through the generosity of Mead Johnson and Company and of Dr. C. E. Bills.

[6] This sample was sent by the Coast Fishing Company of Wilmington, California, through the courtesy of Mr. S. Hornstein to whom we express our gratitude.

TABLE 4

Vitamins D and A in Various Fish Liver Oils

Oil No.	Description	Vitamin D	Vitamin A	Lovibond blue	Vitamin A estimated from L.B.U.
		U.S.P. units per gm.	*U.S.P. units per gm.*	*Units per gm.*	*U.S.P. units per gm.*
5C	California bluefin tuna liver oil extracted by ether from fresh livers.	35,000	78,000	15,000	88,000
5E	California bluefin tuna liver oil extracted by ether from fresh livers.	54,000	.....	11,600	68,000
5G	Hawaiian striped tuna liver oil extracted with ether from fresh livers.	27,000	33,000	6,000	35,000
5A	Tuna offal oil, (California) commercial.	75	203	33	194
5B	Tuna offal oil, (California) commercial.	70	720	107	630
5D	Ether extract of (California) tuna stomachs and contents extracted in laboratory.	35	.....	3,130	18,400
5K	Bluefin tuna liver oil concentrate.	400,000	99,000	15,200	90,000
5ge	Striped tuna liver oil concentrate.	180,000	.....	10,000	60,000
11A	Shark liver oil (Wonder oil), commercial from basking shark.	Less than 35	.....	.....	.....
8A	Shark liver oil, commercial.	Less than 35	12,000	2,200	13,000
8C	Shark liver oil, commercial, made by "Oliver process."	Less than 18	5,500	1,000	5,900
8D	Oil extracted from fresh basking shark (caught in Monterey bay) liver in laboratory.	Less than 18	67	0	0
8E, 8F, 8G	Similar to 8D.	Less than 18	.....	0	0
.....	Reference cod liver oil.	95	3,000	510	3,000
12A	Totoaba liver oil No. I, extracted with ether.	1,400	.....	15,000	88,000
12gd	Totoaba liver oil No. IV, extracted in laboratory after drying.	1,400	45,300	11,600	68,000
12ge	Totoaba liver oil No. IV, extracted with ether.	1,400	70,800	12,500	73,500
12ge	Totoaba liver oil No. IV, concentrate.	10,000	.....	34,000	200,000
12E	Totoaba liver oil No. III, extracted with ether.	3,500	97,400	15,600	92,000
12C	Totoaba liver oil No. II, extracted with ether.	.....	181,000	19,700	116,000
10E	Mackerel liver oil extracted in laboratory from fresh livers.	5,400	211,000	45,000	265,000
10B	Mackerel liver oil extracted in laboratory from fresh livers.	2,700	.....	2,700	16,000
10A	Mackerel oil from cannery refuse, commercial.	75	.....	300	1,700
10F	California mackerel liver oil, 1937 fall catch.	.....	76,000	16,200	95,600
10G	California mackerel liver oil, 1937 fall catch.	.....	30,000	5,000	29,500
14D	Halibut liver oil, medicinal.	940	192,000	43,000	223,000
V-1008	Cod liver oil concentrate.	103,000	.....	40,000	230,000

larger part of these livers was partly dried and extracted with organic solvents at once. Smaller portions were steam extracted or cold extracted and centrifuged. Vitamin D was present in the oil from the fall specimen, 10b, to the extent of 2,700 units per gram, and in the spring liver oil, 10e, at least 5,400 units per gram. The latter oil appeared also to be considerably richer in vitamin A than the fall liver oil, just as might have been expected from the lower fat content of the livers of the fish caught in the spring.

Two additional oil samples made from livers of California mackerel caught in the fall of 1937 were found also to be rich in vitamin A but not equal to the 1935 spring sample.

Black and Sassaman (1936) reported 5,000 and 6,000 units of vitamin D per gram for two samples of mackerel liver oil of Japanese origin, and Bills, *et al.* (1937) found 1,400 units per gram in California mackerel.

VITAMIN CONTENT OF SHARK LIVER OILS

Three commercial and four laboratory specimens of shark liver oil were examined. The commercial oils, 8A and 8C, came from southern California, but neither process of separation nor type of shark was specified. Obviously these oils must have been extracted from the liver of some other kind of shark than that known as "basking" since the several liver oils known to be taken from basking sharks caught in Monterey bay were found negative as to both vitamins, whereas 8A and 8C were relatively rich in vitamin A although poor in D. According to Barnhart (1936) at least 20 kinds of fish belonging to nine different families called "shark" are found in the Pacific ocean. Several of these, such as the mackerel shark, swell shark, gray smooth-hound, leopard shark, bay shark, and dogfish, are more abundant on the southern California coast than the basking shark *(Cetorhinus maximus).*

Our experience is similar to that of Jones and Christiansen (1935) who report that three out of ten shark liver oils were found rich in vitamin A, seven poor in vitamin A, and all poor in vitamin D.

DISCUSSION OF METHODS OF VITAMIN A ASSAY

The comparison of vitamin A assays of all these fish oils by the colorimetric and biological methods is of interest. The U.S.P. reference cod liver oil was used as a check on the color production and constancy of reading in all the colorimetric tests and the approximate number of units per gram of oil was estimated for the unknowns by direct proportion of blue unitage of the reference and the unknown oils to the known vitamin A content of the former. Striped

tuna liver oil 5G, for example, has 6,000 Lovibond blue units per gram and the reference oil 510. The assigned vitamin A potency of the reference oil is 3,000 units per gram. The striped tuna liver oil had, therefore, approximately $\frac{6,000}{500}$ x 3,000 or 35,000 units of vitamin A per gram. Emmett, Bird, Neilsen, and Cannon (1932) used a similar calculation in their study of halibut liver oil.

Most of the difficulty in this method lies in obtaining dilutions which give the same depth of blue color within the proper range of tintometer readings. Readings below four and above seven appear to be of doubtful significance and all readings should be made at five to be comparable. Thus all the sardine oil readings which were under three yield no more than evidence of a trace of the vitamin, and for this reason vitamin A values in units per gram are not calculated from them. Since oil concentrations above 15 to 20 per cent have been shown by Drummond and Hilditch (1930) to produce irregular and non-predictable blue values with the antimony trichloride reagent, the range of vitamin content necessary for use of this method can be fairly well outlined. If the blue readings be kept close to five and oil concentrations at or below 20 per cent, the minimum blue reading to be used represents 125 blue units per gram; and, by analogy with the reference cod liver oil, the minimum value for the unknown is about 750 units per gram. On the other hand, there is no limit apparently to the amount of dilution which may be utilized in obtaining suitable color readings with extremely potent oils.

The question of the quantitative reliability of the expression of vitamin A in units has been discussed too frequently to need repetition here. Everyone familiar with the biological method is conscious of the hazards involved in any attempt at fine distinctions based on its use. The tintometer method has limitations still more obvious. With continued experience in the use of both methods, nevertheless, one accumulates a certain confidence in the major distinctions obtained. The use of the antimony trichloride reaction for the determination of the levels at which oils are fed for the biological assay is economical and satisfactory.

The calculation of number of units of vitamin A per gram by the biological method was carried out as suggested in the official U.S.P. XI directions. Every assay involved the feeding of one or more animals from each litter a dose of reference cod liver oil found by tintometer to be about equal in blue value to the dose of unknown oil fed at the same time to other members of the litters. Usually 16 to 19 mg. of the reference oil was found sufficient to allow 24 to 36 grams gain in weight in vitamin A-deficient rats in the four weeks

of observation. The growth of litter-mate rats on the unknown during the same time was taken to indicate the relative vitamin A content of the unknown.

SUMMARY

Only one out of 14 commercial sardine oils of the 1934 catch was found to meet the minimum vitamin D standard for U.S.P. cod liver oil. All had negligible amounts of vitamin A. Both methods of separation and lapse of time before separation affected the vitamin content of sardine oils. Eight out of 11 spot and composite samples taken from one large run in the late 1938 season were at least equal to the U.S.P. reference cod liver oil in vitamin D content and contained more than 100 Lovibond blue units per gram. The latter catch yielded oil three to four times as rich in both vitamins as did the 1934 catch.

Seven commercial salmon offal oils contained 58 to 142 units of vitamin D per gram, two pink salmon oils being the richest in this factor. The vitamin A of four of these oils approached the minimum standard for U.S.P. cod liver oil.

Dog salmon (keta) liver oil had about one-tenth (5,800 units per gram) the vitamin A of silver salmon (coho) liver oil (57,600 units per gram) although the two had nearly the same vitamin D content, about 500 units per gram.

Totoaba (white sea bass) livers were found to be rich in oil which contained 1,400 to 3,500 units of vitamin D per gram and 45,000 to 180,000 units of vitamin A per gram, in both cases similar to the range found usually in halibut liver oil.

Three laboratory extracted tuna liver oils were found to contain 35,000 and 54,000 units of vitamin D per gram, and a somewhat larger content of vitamin A. Tuna offal oils were of low vitamin content.

Two lots of freshly extracted mackerel liver oil had 2,700 and 5,400 units of vitamin D per gram and 16,000 and 211,000 units of vitamin A. Two later samples, fall catch 1937, had 76,000 and 30,000 units of vitamin A per gram. The oil content of these livers was in inverse relation to the vitamin value of the oils.

The liver oil of the basking shark was found on repeated examination to be nearly devoid of both vitamins A and D, but two commercial shark liver oils, possibly from another kind of shark, were relatively rich in vitamin A although poor in vitamin D.

The conclusion is reached that there is no predictable relation between the vitamin A and D contents of these fish oils nor between oil content of the livers and vitamin value of the oils.

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SEASONAL VARIATION IN CHEMICAL COMPOSITION OF COMMON HADDOCK[1]

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We are told by Pierce (1934) that until about 1870 the New England fishermen threw back into the sea the haddock which they accidently caught while fishing for the then much more highly valued cod. This is a far cry from the present when, according to official statistics of the United States Bureau of Fisheries (1935), 261,653,000 pounds of haddock were unloaded at American seaports in 1929 with a value of $9,142,000 to the fishermen of the United States. This amount does not include the additional labor supplied to the large number of men employed in handling the fish from the time it was unloaded from the boats until it reached the consumer.

Since that time an appreciable scarcity of haddock has occurred as evidenced by an increased market price. Whether this is due to the exhaustive fishing of the waters frequented by the haddock or to the methods used is not a problem for discussion in this paper, but is introduced merely to show the importance of the fish as a food product.

The purpose of this investigation was to determine the chemical composition of haddock frozen by various methods and at different seasons of the year, thus bringing information on the subject to a point approaching that known about some of the other American food fishes.

REVIEW OF LITERATURE

Among the earliest investigators to make a really thorough study of the chemical composition of fish was Atwater (1892) who published the results of several years work covering the analysis of 52 species of common American fishes, two species of European origin, and 11 species of American mollusks and crustacea. In that work he determined the amounts of water, ash, ether extract, and total nitrogen present. From these he calculated the food value using the familiar values of 4.1 calories per gram for protein and carbohydrate and 9.3 calories per gram for fat. In addition a limited amount of

[1] Data taken from a thesis presented to the Graduate School, Massachusetts State College, June, 1937, in partial fulfillment of the requirements for the Ph.D. degree. Contribution No. 314.

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work was also done on the digestibility and retention of nutritive materials from the fish, using a man and a dog as subjects.

Smith (1913) was among the early workers to study the effect of cold-storage temperatures on the chemical composition of fish. He compared the amounts of water, total solids, organic matter, ash, ammonia, and fat found in common flounder muscle when fresh and after storage periods of six and nine months. From his data one can only conclude that there was no significant change in any of the constituents for which analyses were made.

Following somewhat the same line of work Clark and Almy (1920) studied the effect of cold storage on bluefish and whitefish. They concluded that there was a slight increase in the amino acid and ammonia content of the muscle during storage. At the same time an increase in the acid value and a decrease in the iodine number were observed for extracted fat.

Reay (1930) started a series of papers covering a rather extensive study of the effect of freezing on the protein of fish muscle. In the first of his papers he concluded that the change may be largely the result of desiccation with the resulting change in the concentration of the salt solution of the cells, in turn, affecting the proteins.

The following year Lemon (1931) published a paper based on attempts to prevent desiccation of frozen fish by coating them with a glaze of ice, cottonseed oil, corn oil, peanut oil, and hydrogenated cottonseed oil. All of these allowed a loss of moisture ranging from 5½ to 23 per cent of the water present. These rather large losses may be partly explained by the fact that in the experiment he used a storage temperature of —9.4°C.(15°F.) as compared with the now more common holding temperature of —17.8 to —20.6°C.(0 to —5°F.) for frozen fish, fruits, or meats.

Later Reay (1933) again attacked the problem from the standpoint of protein denaturation. He found that the most rapid change in the muscle could be expected between —2 and —4°C.(28.4 to 24.8°F.). His work covered temperatures from 0 to —20°C.(32 to —4°F.).

The following year Finn (1934) published the results of similar studies on the juice extracted from fish muscle. He too found that at —2°C. the denaturation of the proteins was very rapid with about 24 per cent of the total undergoing change in 84 days, while at —20°C. only three per cent was denatured in the same period or time. He too raised the question of the changing water content of the muscle at different temperatures as a possible explanation of the problem.

In 1935 similar studies were made by Reay (1935) but with the idea of preserving fish in the best possible condition for smoke curing. This same work was later discussed by Lumley (1936).

The entire subject of fish freezing and the changes produced has been ably reviewed and discussed by several writers, especially Finn (1936), Tressler (1933), and Tressler and Evers (1936).

(In this connection it should also be mentioned that a mimeographed bibliography, containing about 180 references dealing with the whole field of frozen foods, has been prepared for distribution by the Birds Eye Laboratories of Boston, Massachusetts.)

Greene (1919) made a study of the variation in fat and protein content of the king salmon during the spawning migration. Similar work was done by Davidson and Shostrom (1935) on the pink salmon. Both of these were prompted by a desire to determine the optimum time and conditions for catching and canning these two valuable food fishes.

The question of seasonal variation in chemical composition of fish was again mentioned by Reay (1936) when he found the fat content of herring varying from about one to 25 per cent. This is also undoubtedly closely associated with the feeding and migratory habits of this species.

Some work has also been done in studying the mineral content of certain fish. The iron content of several species of fish has been studied by Peterson and Elvehjem (1928) and Toscani and Reznikoff (1934) and found to vary considerably.

Peterson and Skinner (1931) determined the amount of manganese in a number of fishes and crustacea but reported no figures for haddock. Lindlow and Peterson (1927) analyzed halibut and bluefish for the same element but found none present.

Lindow, Elvehjem, and Peterson (1929) determined the amount of copper in a number of foods including haddock. Parks and Rose (1933) examined about 20 species of fish for copper, iron, and manganese in an attempt to compare the composition of fresh and salt water fishes.

Among the rarer elements Bodansky (1921) analyzed certain varieties of fish for zinc, while Meulen (1932) reported .03 milligram of molybdenum per kilogram of whole fish.

Several workers have studied the ammonia content as a decomposition product of the nitrogenous material. These references are discussed in considerable detail by Crooks and Ritchie (1938) in another paper devoted entirely to a study of the ammonia content of haddock muscle and its relation to the state of preservation of the sample.

DESCRIPTION AND PREPARATION OF SAMPLES

In this problem four series of samples of haddock were frozen at intervals of approximately one month, extending over a period of 11 months. The four series of samples will be referred to in the following pages as Series B, Series C, Series D, and Series E.

All of the fish were caught by commercial trawlers of the General Sea Foods Corporation. The fish designated as Series B were eviscerated as soon as caught, wrapped in parchment, and immediately frozen by being placed with solid carbon dioxide in an insulated chest. This chest was so constructed and of such size that it would hold four 50-pound cakes of solid carbon dioxide. It was found that if the chest were not opened until about the last day of fishing, or about six or seven days after leaving port, only about one-third of the dry ice had evaporated. When the chest again arrived in port there was always enough of the solid carbon dioxide left to keep the fish solidly frozen. These fish were then transferred to one of the holding rooms at the Boston Fish Pier where they were kept frozen until they were shipped to the laboratory in solid carbon dioxide.

Series C consisted of fish commercially handled until reaching the Boston Fish Pier; that is, the fish were caught and eviscerated by the same means employed for Series B. They were packed in chipped ice in the hold of the vessel until unloaded at the pier. Here they were dipped in a sodium chloride brine, wrapped in parchment, and frozen whole in a Birds Eye multiplate freezer. They were stored in one of the holding rooms at the Boston Fish Pier until shipped to the laboratory at Amherst, packed in solid carbon dioxide.

Series D and Series E were commercial haddock fillets. Series D was prepared according to the procedure followed by the packers of the Birds Eye Frosted Foods. These methods have been discussed in detail by Lemon (1932). The fish were filleted within two or three hours after unloading from the boat. The fillets were dipped in a 35° salimeter sodium chloride solution for about 22 seconds. After being wrapped in parchment the samples were packed in the regular commercial five-pound cartons and frozen in a Birds Eye multiplate freezer. About 30 minutes were required for freezing before the cartons were packed in solid carbon dioxide for shipment to the laboratory.

Series E was prepared in the same way except for the method of freezing. The cartons were placed in a freezing room, according to the procedure of the sharp method, at a temperature of about —34.5°C.(—30°F.). This required 18 to 24 hours for complete freezing.

The season of the year that each sample was frozen will be designated in this paper by the use of exponents above the number of the sample. Ten samples of each series were frozen at intervals of a little over a month extending from August 20, 1935, to July 10, 1936. Thus, the four samples frozen on August 20, 1935, will be spoken of as Samples B^1, C^1, D^1 and E^1, respectively; while the samples frozen on September 20, 1935, will be designated as Samples B^2, C^2, D^2, and E^2. Following this system the samples on July 10, 1936, will be referred to as Samples B^{10}, C^{10}, D^{10}, and E^{10}, respectively.

EXPERIMENTAL PROCEDURE

Water Content

In view of the amount of previous work done by Lemon (1931) and Tressler (1933) on the problem of the water content and the prevention of desiccation of frozen fish, any further study on that subject would have been superfluous. The possibility of slight seasonal variation, however, and the need of knowing accurately the water content of the samples in order to calculate the percentage composition of other constituents on both fresh and dry bases made it seem advisable to determine the moisture in representative samples of fish frozen by each of the four methods used in this work, and from each catch, thus covering a period of approximately one year.

As soon as the samples reached the laboratory, frozen in every case, representative samples of each, free from bones and skin, were, without defrosting, finely ground with a hand meat chopper, introduced into weighed weighing bottles, and dried to constant weight in a vacuum oven at 55°C.(131°F.). This temperature was used because, although it was high enough to give constant weight in from 24 to 36 hours, it was not high enough to perceptibly affect the small amount of oil in haddock muscle.

Following this procedure, results obtained (Table 1) will show that there was no significant difference in the water content of samples frozen by the four different methods nor any significant seasonal variation as found in the muscle of some species.

Total Ash

As a means of determining the relative losses of soluble salts from the samples processed by the four methods previously described, it seemed advisable to determine the amount of total ash in all of the samples received. To do this, samples which had been vacuum-dried at 55°C.(131°F.) in weighing bottles were weighed and introduced into small platinum dishes. The amount used was then calculated by difference. The samples were ashed to constant weight in an electric

muffle oven set to maintain a very dull redness. The results of these determinations are given (Table 2).

One will notice at once that samples of Series B and Series C show considerably lower ash contents than do Series D and E. This can be readily explained in the light of the commercial practice of

TABLE 1

Water Content of Haddock Muscle

Date of sample	Series B average	Series C average	Series D average	Series E average
	pct.	*pct.*	*pct.*	*pct.*
Aug. 20, 1935	80.77	81.21	80.96	80.40
Sept. 20, 1935	80.60	81.64	80.39	80.35
Oct. 24, 1935	79.28	80.24	80.65	80.61
Dec. 4, 1935	[1]	80.44	79.15	79.70
Dec. 22, 1935	80.40	80.02	79.89	79.98
Feb. 10, 1936	80.24	80.44	79.49	79.58
Mar. 12, 1936	80.84	79.87	80.61	80.62
Apr. 10, 1936	80.11	81.51	81.61	79.77
May 6, 1936	84.12	81.09	81.71	82.89
July 10, 1936	82.73	79.68	81.06	80.58
Averages	81.01	80.61	80.55	80.45

[1] Samples lost at sea.

TABLE 2

Ash Content of Haddock Muscle (Dry Basis)

Date of sample	Series B average	Series C average	Series D average	Series E average
	pct.	*pct.*	*pct.*	*pct.*
Aug. 20, 1935 [1]	6.61	6.87	7.14	7.45
Sept. 20, 1935 [1]	6.34	6.27	9.26	10.31
Oct. 24, 1935	6.00	6.88	7.19	7.04
Dec. 4, 1935	[2]	5.94	8.05	8.41
Dec. 22, 1935	6.26	5.75	6.98	6.83
Feb. 10, 1936	6.28	6.33	8.04	8.00
Mar. 12, 1936	6.45	6.23	7.49	7.61
Apr. 10, 1936	6.30	5.88	8.48	8.60
May 6, 1936	7.05	7.20	8.52	8.64
July 10, 1936	7.30	6.57	7.35	7.06
Averages	6.51	6.39	7.85	7.80

[1] Determinations made on fresh samples and calculated to dry basis. Other determinations on vacuum-dried material. [2] Samples lost at sea.

"brining" the samples before freezing. In the process the pieces of fish (whole in the cases of B and C and fillets in the cases of D and E) are lowered into a barrel of sodium chloride brine for about 22 seconds. This brine is made up to give a salimeter reading of 35°. Thus the amount of salt which penetrates the muscle is influenced

considerably by the thickness of the piece "brined" and by any variation which may be allowed to take place either in the concentration of the brine or in the time the samples are allowed to remain in it.

Ether Extract

Following the method prescribed by the Association of Official Agricultural Chemists (1930b) fat, or more accurately the ether extract, was determined in all samples, not because it was anticipated that there would be any great difference in the samples processed by the four methods previously described, but because there has been some indication that there may be a seasonal variation in the amount of fatty material present in the muscle of certain fish. A survey (Table 3) will show that, although the haddock is very low in ether soluble

TABLE 3

Ether Extract of Haddock Muscle (Dry Basis)

Date of sample	Series B average	Series C average	Series D average	Series E average
	pct.	*pct.*	*pct.*	*pct.*
Aug. 20, 1935	.750	.630	.620	.770
Sept. 20, 1935	1.345	.935	.770	.620
Oct. 24, 1935	.710	.720	.530	.675
Dec. 4, 1935	[1]	.470	.640	.590
Dec. 22, 1935	.420	.435	.495	.520
Feb. 10, 1936	.550	.515	.605	.585
Mar. 12, 1936	.560	.550	.590	.590
Apr. 10, 1936	.630	.540	.575	.675
May 6, 1936	.550	.590	.500	.490
July 10, 1936	.435	.550	.580	.630
Averages	.661	.594	.591	.615

[1] Samples lost at sea.

material at all times, there is a slight rise during the late fall, followed by a rapid decline during December that continues until late in the summer.

Total Organic Nitrogen

Recognizing the fact that fish muscle is a valuable food, primarily because of its high protein content, it seemed important to determine whether the common methods of freezing altered the amount of this constituent to an appreciable extent.

With this end in view, the total organic nitrogen of all samples was determined, following the method prescribed by the Association of Official Agricultural Chemists (1930c) for meat products.

Results of these analyses show that there was a striking uniformity not only among the samples frozen by the various methods but also

among the samples taken throughout the year (Table 4). Considerable difficulty was experienced in obtaining perfectly uniform samples free of very small bones. This is believed to account for any variations noted. To overcome this difficulty as completely as possible, after the first two samples (Aug. 20 and Sept. 20, 1935) very large samples were dried, very finely ground, remixed, sampled, and dried *in vacuo* before being analyzed.

Ammonia

As a result of the extensive work of Lucke and Geidel (1935), Tillmans and Otto (1924), and Tillmans, Hirsch, and Kuhn (1927) it is generally accepted that the ammonia content of fish or meat bears

TABLE 4

Organic Nitrogen Content of Haddock Muscle (Dry Basis)

Date of sample	Series B average	Series C average	Series D average	Series E average
	pct.	*pct.*	*pct.*	*pct.*
Aug. 20, 1935	16.64	16.42	16.36	16.64
Sept. 20, 1935	16.08	15.99	15.68	14.25
Oct. 24, 1935	15.23	15.04	15.06	15.08
Dec. 4, 1935	[1]	15.48	14.92	14.91
Dec. 22, 1935	15.50	15.50	15.14	15.09
Feb. 10, 1936	15.65	15.58	15.14	15.22
Mar. 12, 1936	15.55	15.60	15.38	15.38
Apr. 10, 1936	15.63	15.59	15.09	14.99
May 6, 1936	15.04	15.13	14.71	14.81
July 10, 1936	15.15	15.04	14.15	14.44
Averages	15.61	15.54	15.16	15.08

[1] Samples lost at sea.

a rather definite relation to the state of preservation of their nitrogenous component parts. Thus, since in this study we are interested in the relative merits of certain methods of freezing fish muscle for human consumption, it seemed advisable to determine the amount of ammonia in all samples as a means of checking the quality of fish frozen by the four methods previously outlined.

Using a modified Folin aeration method as prescribed by the Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists (1930a), results were obtained as shown (Table 5).

It will be noted that there was practically no free ammonia present in any of the regular samples. In one case, one sample showed an ammonia content of .26 milligram per gram of fresh muscle. This one sample, however, had almost twice as much ammonia as any other similar sample, thus rather definitely indicating that

somewhere in processing or storage, that sample had not retained its freshness as completely as had the others.

Inorganic Constituents

Owing to the rather extensive work which has been done by previous workers on the inorganic constituents of fish in general, and since there is no reason to believe that these constituents would be affected by freezing or storage, the present work along that line was limited to analysis of the dried muscle for copper, iron, manganese, and phosphorus.

TABLE 5

Ammonia Content of Haddock Muscle [1]

Date of sample	Series B average	Series C average	Series D average	Series E average
Aug. 20, 1935	.000	.050	.000	.050
Sept. 20, 1935	.100	.075	.141	.145
Oct. 24, 1935	.100	.135	.100	.200
Dec. 4, 1935	[2]	.078	.088	.100
Dec. 22, 1935	.090	.125	.085	.085
Feb. 10, 1936	.090	.090	.095	.135
Mar. 12, 1936	.015	.045	.035	.035
Apr. 10, 1936	.080	.140	.120	.130
May 6, 1936	.046	.074	.064	.064
July 10, 1936	.056	.077	.085	.085
Averages	.064	.089	.081	.103

[1] Given in milligrams per gram of fresh muscle. [2] Samples lost at sea.

As in the previous work, four sets of samples were compared in each case and identified as before. Samples of each of these were taken at four different periods in order to divide the data roughly with respect to the four seasons of the year.

Copper

The method of analysis used was developed in this laboratory by Dr. Edward B. Holland and Mr. R. A. Coughy,[3] based on earlier work by Callan and Henderson (1929), Elvehjem and Lindow (1929), McFarlane (1932), Mosely, Rohwer, and Moore (1934), and Sylvester and Lampitt (1935). This is a colorimetric method using the color developed by the addition of a solution of sodium diethyl-dithiocarbamate to the clear solution obtained by digesting the dried sample with sulphuric and nitric acids.

The results of these analyses (Table 6) compare very favorably with analyses reported by Lindow, Elvehjem, and Peterson (1929) in which they give a copper content of .00134 per cent calculated on a

[3] Personal communication.

dry basis. Parks and Rose (1933), when making a study of the copper, iron, and manganese content of certain Lake Champlain fishes, analyzed a few haddock in order to compare fresh and salt water fishes. They reported 2.3 milligrams of copper per kilogram of fresh fish muscle. This on a basis of the moisture content which they reported as 79.1 per cent, for the same samples, may be calculated to

TABLE 6

Copper Content of Dry Haddock Muscle

Date of sample	Series B	Series C	Series D	Series E
	pct.	*pct.*	*pct.*	*pct.*
Aug. 20, 1935	.00278	.00072	.00064	.00106
Oct. 24, 1935	.00256	.00141	.00098	.00124
Dec. 22, 1935	.00084	.00087	.00106	.00101
Mar. 12, 1936	.00066	.00075	.00073	.00075
Average	.00171	.00094	.00085	.00102

give a copper content of .00110 per cent on a dry basis.

In neither of these reports was any mention made as to the season of the year during which the fish were caught. A study (Table 6) will show that in all samples the amount of copper found in the muscle was low after the spawning migration in February or early March.

Iron

Analyses for iron were made on the same ash filtrates that were used for the copper determinations, following a method developed in

TABLE 7

Iron Content of Dry Haddock Muscle

Date of sample	Series B	Series C	Series D	Series E
	pct.	*pct.*	*pct.*	*pct.*
Aug. 20, 1935	.00873	.00502	.00413	.00630
Oct. 24, 1935	.00390	.00351	.00415	.00368
Dec. 22, 1935	.00310	.00270	.00280	.00358
Mar. 12, 1936	.00209	.00198	.00186	.00176
Average	.00446	.00330	.00324	.00383

this laboratory based on the earlier work of Kennedy (1927), Peterson and Elvehjem (1928), and McFarlane (1932).

The results of these analyses (Table 7), as in the previous work on copper, show that there is no appreciable difference in the iron content of the samples frozen by the various methods. The samples average more iron than was reported by Peterson and Elvehjem (1928) and Parks and Rose (1933). The former workers reported

only .00042 per cent while the latter reported 4.8 milligrams of iron per kilogram of fresh muscle. Again calculating on the basis of their reported 79.1 per cent of water, this figure can be calculated to give .00230 per cent of iron on a dry basis.

There is some evidence of a seasonal variation which is considerably greater than any difference observed between samples of the same catch but frozen by a different method. It will be noted that the low iron analyses are also observed following the spawning migration.

Manganese

The determination of manganese in vacuum-dried haddock muscle followed a method based on the work of Reiman and Minot (1920), Willard and Greathouse (1917), Richards (1930), Lindow and Peterson (1927), Skinner and Peterson (1928), and Bolin (1934).

One hundred milliliters of the original ash solution were evaporated to dryness on a steam bath with five milliliters of concentrated nitric acid, taking advantage of the color obtained by oxidizing the manganese present to the permanganate with potassium periodate.

In this work the extremely small amount of manganese present did not give enough color to make a color comparison against a standard possible. A distinct difference was noted, however, with a maximum color produced in the samples of October 24, decreasing through December to give a minimum in March, followed by a slight increase in August. Using a very similar method, Lindow and Peterson (1927) reported no manganese in halibut or bluefish, while Peterson and Skinner (1931) reported manganese contents of .0063 per cent for cod and red snapper. Very little work seems to have been done on the manganese content of fish muscle and no perfectly agreeing data have been found for the manganese in haddock.

Phosphorus

Phosphorus was determined in the same four representative samples, using essentially the method of Fiske and Subbarow (1925). There was no significant variation in the phosphorus content either among the samples frozen by the various methods or among samples taken at different seasons of the year (Table 8).

Analyses were also made to determine the effect of storage upon the amino acid content of the tissues and upon the nitrogen fractions. This work is being continued and will be published at a later date.

SUMMARY

Water, ash, ether extract, organic nitrogen, ammonia, copper, iron, manganese, and phosphorus were determined on four series of

haddock samples caught over a period of one year. These four series of samples included fish frozen whole at sea by means of solid carbon dioxide as soon as caught, fish frozen whole by the Birds Eye method as soon as landed and commercial fillets frozen by both the Birds Eye and sharp methods. No significant differences were found in the composition of samples frozen by the various methods, although there is a suggestion of some seasonal variation in certain constituents.

TABLE 8

Phosphorus Content of Dry Haddock Muscle

Date of sample	Series B	Series C	Series D	Series E
	pct.	*pct.*	*pct.*	*pct.*
Aug. 20, 1935	1.1267	0.9786	1.0000	0.9412
Oct. 24, 1935	1.1429	1.1726	0.9413	0.9303
Dec. 22, 1935	1.0666	1.0666	1.0000	1.0204
Mar. 12, 1936	1.0423	1.0000	0.9581	0.9877
Average	1.0946	1.0545	0.9749	0.9699

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STUDIES ON PRODUCTION OF ETHYLENE IN THE RIPENING PROCESS IN APPLE AND BANANA

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The effects of ethylene on plant tissues have long been known and, in their externals, extensively studied. These studies began when Neljubov (1901) observed that low concentrations of ethylene in air caused inhibition of elongation together with bending and swelling responses in pea seedlings. Harvey (1915), also working with pea seedlings, described effects of ethylene on the respiration and chemical composition of the tissues. These studies of growth responses produced by ethylene have been greatly extended by Crocker, Hitchcock, and Zimmerman (1935).

Denny (1924) showed that ethylene, in concentration of 1:10,000 in air, greatly accelerated the removal of chlorophyll from the peel of lemons, and Harvey (1926) discovered the effect of ethylene in hastening the ripening of fruits.

Later, Botjes (1933), Elmer (1932), and Huelin (1932) obtained evidence of the presence of a gas of high physiological activity in the emanations from apples, and Gane (1934) identified this gas as ethylene.

The presence in a fruit of a gas of the high biological activity of ethylene is of extreme interest to the physiologist but, owing to the lack of a suitable analytical method for the determination of ethylene in the minute quantities in which it occurs, little quantitative research has been done on its mode of production and action in the fruit. This paper describes a method for determining ethylene quantitatively in fruits and presents some data obtained by the use of this method which bear on the fundamental questions as to the function of ethylene in the economy of plants.

DETERMINATION OF ETHYLENE IN PLANT TISSUES

Attempts have been made to estimate the quantity of ethylene produced by apples. Gane (1934) passed the emanations from 60 pounds of apples over bromine at —65°C.(—85°F.) for four weeks and was able to estimate from the weight of ethylene bromide isolated that "the amount of ethylene produced was very small, perhaps of the order of 1 c.c. during the whole life history of the fruit."

Later Denny and Miller (1935) confirmed this estimate by experiments determining the minimum weight of apple tissue which would produce epinasty in a potato plant in a bell jar of known capacity.

Obviously such methods are unsuited for exact determinations, and furthermore, they measured only the amount of ethylene produced in a long period of time and not the ethylene content of the tissue at a given moment.

In order to do this last, it is necessary to extract the gases present in the tissue, remove from them all substances which will interfere with the determination, and cause the ethylene remaining to react in such a way as to produce the greatest and most easily measurable effect.

Evidently the most useful reaction of ethylene for the purpose is its power of reducing dilute potassium permanganate solution, since the extent of this reaction can be measured readily by pipetting an

TABLE 1

Standardization of Procedure Against Ethylene

Vol. C_2H_4	Vol. $KMNO_4$	Cal. Vol. C_2H_4	Theor. Oxid.
			pct.
.0240	0.92	.0252	105
.0249	0.95	.0273	110
.0338	1.29	.0357	106
.0353	1.35	.0378	107
			Aver. 107

aliquot of permanganate into excess ferrous solution and titrating back with permanganate. If the titration be carried out in a long tube, .03 ml. of .002 N permanganate solution will produce a detectable pink coloration, a sensitivity sufficient for the purpose.

Ethylene is only very slowly absorbed by dilute acid permanganate solution. This is due to the fact that ethylene has only a slight water solubility, and furthermore, the reaction between ethylene and dilute permanganate is relatively slow. After several attempts, an absorber of great enough efficiency was devised and described by Nelson (1937).

This mode of oxidation of permanganate, while capable of yielding good results under ideal conditions, has been found to be too cumbersome and difficult of operation to be entirely practicable. The absorber is difficult to clean and dry, and the stopcock creates a continual hazard of getting grease into the absorber where it may reduce permanganate. Therefore, after many trials, it was abandoned and a new method adopted.

In the new procedure the ethylene is introduced into an evacuated bulb where it is oxidized by dilute permanganate. The bulb is rotated mechanically in order to present a large absorbing surface. The form of apparatus finally adopted is shown (Fig. 2). The elimination of stopcocks makes it possible to run perfect blanks consistently with this apparatus, and it is easy to clean and dry.

When this procedure was standardized against pure ethylene, it was found that .030 c.c. of ethylene reduced 1.13 ml. of .002 N permanganate which was 107 per cent of theoretical oxidation to glycol calculated on the basis that the manganese dioxide formed remained in suspension and that an aliquot portion of it was removed.

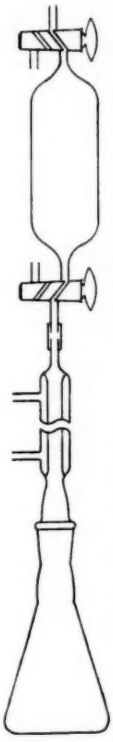

FIG. 1.

For the removal of interfering substances a number of reagents were tried. Soda lime was found to remove acetaldehyde quantitatively from a stream of gas. Anhydrous aluminum chloride was effective in removing both alcohols and ethylene. Finally, a single reagent, sodamide, was found which removed from the air stream all permanganate reducing substances present in apples other than ethylene.

There is some hazard connected with the use of sodamide owing to the occasional formation of explosive compounds with air, and all due precautions should be observed. Sodamide also reacts violently with water. However, sodamide, which had been in use for six months and constantly exposed to air for that time, was cautiously recrushed without accident, and it would seem that its use was justified, particularly in view of the fact that it is the only thing which makes the determination possible.

Experimental Procedure

The apparatus used for the extraction of ethylene from plant tissues consists of a 500-ml. Erlenmeyer flask which is fitted by means of a 25-mm. ground joint to a short reflux condenser (Fig. 1). To the upper end of the reflux condenser, a Pyrex gas-sampling bulb of 330-c.c. capacity is attached with rubber tubing.

The gases extracted from the tissue are freed from interfering substances by passing through the train shown (Fig. 2). The tube is packed successively with a layer of Dehydrite four inches long, one of sodamide six inches long, and another of Dehydrite one inch long. The sodamide of commerce is crushed to pieces of about one

mm. size under toluene, placed in the tube while still wet, and freed of toluene by passing air through the fully loaded tube.

At C is shown the vessel in which the ethylene is oxidized by dilute permanganate solution. It was constructed from a 1 L Pyrex balloon flask and standard taper ground joints. The side arm is closed by a short piece of rubber tubing fitted with a Hoffman screw clamp.

The device for agitating the permanganate solution while oxidation of the ethylene is taking place (Fig. 3) was constructed from odds and ends found around the laboratory and rotates the flask at

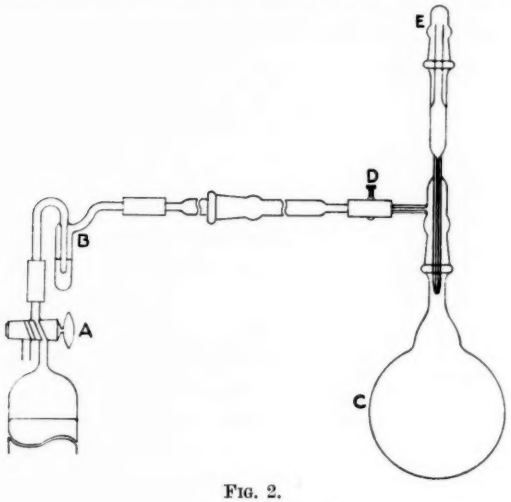

FIG. 2.

the rate of 80 turns per minute. The flask is held in place with rubber binders.

Two hundred milliliters of water are placed in the Erlenmeyer extraction flask and it is stoppered and weighed. The sample is placed in the flask and it is again weighed, the weight of the sample being obtained by difference.

Fleshy fruits, such as apples and bananas, are sampled with corkborer of 20 mm. diameter, and the plugs of tissue so taken are expelled directly into the flask. Sampling of apple tissue is facilitated if the corkborer is first pressed into the fruit around the core and withdrawn, leaving the core in place. This makes it easy to remove the plugs which constitute the sample. In any case, sampling is performed as rapidly as possible to avoid unnecessary loss of ethylene.

The flask containing the sample is then connected with the reflux condenser, and to the condenser is connected the gas-sampling bulb

which previously has been evacuated. The lower stopcock of the gas-sampling bulb is then opened, placing the whole system under reduced pressure. Water is passed through the reflux condenser, and the contents of the flask are boiled until all the air held in the tissue has been expelled.

With apple tissue it has been found that the following procedure expedites the extraction. When the tissue and water are first heated, the expansion of the air in the tissue causes it and the water to fill the whole flask before the temperature has been raised to the boiling point. When this occurs, the contents of the flask are cooled to their original volume and then reheated, this process being repeated until it is possible to boil the water. Then the reflux condenser is drained and the water boiled until all the air in the system has been expelled into the gas-sampling bulb. The stopcock on the bulb is then closed and water admitted cautiously into the condenser. This lowers the

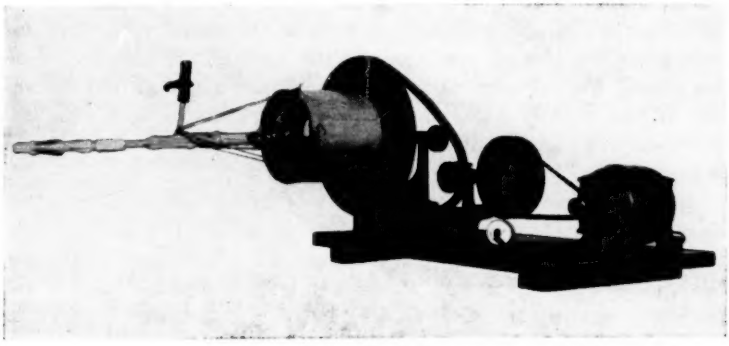

FIG. 3.

pressure in the flask, which facilitates the removal of air from the interior of the plugs of tissue. When the water and tissue have cooled somewhat, the stopcock of the sampling bulb is opened and the process repeated until the tissue has become translucent. Care must be taken not to cool the water and tissue too much while the connection to the sampling bulb is closed, or the flask may be broken.

When the tissue is translucent, the connection with the sampling bulb is opened and the water in the flask is boiled until all the air has been expelled into the bulb, after which the bulb is disconnected from the boiling apparatus.

The sampling bulb now contains all the ethylene present in the tissue together with most of the alcohol, acetaldehyde, and esters. These interfering substances are removed by passing over sodamide.

The purifying train is assembled as shown (Fig. 2), the gas-sampling bulb being placed at one end and the evacuated reaction vessel at the other. First, the lower stopcock of the gas-sampling bulb is opened and its contents are placed under a pressure of about an inch of mercury. Then the upper stopcock A is opened, and the train is ascertained to be free from leaks by observation of the bubble counter B. Now the whole train up to the screw clamp D is under a small pressure. Finally the screw clamp is opened, and with it the flow of gas through the system is adjusted to 10 to 12 c.c. per minute. This rate is maintained by keeping the pressure in the sampling bulb at approximately the same level by adjusting the height of the mercury-leveling bulb.

When the contents of the sampling bulb have been passed through, the train is washed out with air at the same rate for ten minutes, at the end of which time the screw clamp is closed and the reaction vessel is disconnected. The pressure in the vessel is brought up to atmospheric by removing the cap E from the vessel, and three seconds after the hissing produced by the rush of air into the vessel has ceased, five ml. of dilute permanganate are pipetted into the vessel. If this rule is followed, the permanganate will be drawn into the vessel completely but quietly and without spattering. The vessel is then rotated for an hour, as shown (Fig. 3).

The dilute permanganate solution was freshly prepared by pipetting two ml. of .1 N permanganate into 20 ml. of .1 N sulfuric acid.

The extent of reduction of permanganate is measured as follows: Five ml. of .0085 N ferrous ammonium sulfate solution in .1 N sulfuric acid are pipetted into an 18 x 150 mm. test tube. Four ml. of the remaining dilute permanganate are pipetted into this, and two drops of 85-per cent phosphoric acid are added. This is titrated with .002 N permanganate solution freshly prepared by dilution of .1 N permanganate with .1 N sulfuric acid to a pink coloration.

A four-ml. aliquot of the permanganate in the reaction vessel is treated in like manner, and the difference between the titration of the control permanganate and that used to oxidize ethylene is taken as a measure of the ethylene content of the tissue.

The reaction vessel must be kept scrupulously clean and dry, and after use, it is washed first with ferrous sulfate in sulfuric acid and finally with chromic-sulfuric acid cleaning solution strong enough to remove stopcock grease in the cold. Drying is best done in an oven.

ETHYLENE CONTENT OF STORED APPLES

The fact that the apple has the longest storage life of all fresh fruits, together with the fact that the apple also produces more eth-

ylene than any other tissue known, makes a study of the relation between keeping quality and ethylene content of apples of interest.

Six varieties of apples, all commonly grown in Minnesota and having widely different lengths of storage life, were selected. Of these, Wealthy and Patten Greening have a very limited life in storage; that of McIntosh is appreciably longer; and it, in turn, is exceeded in this respect by Malinda, Haralson, and Northwestern Greening, in order of increasing storage life.

Experimental Procedure

Apples of these varieties were obtained from the University of Minnesota Fruit Breeding Farm and from the market. Fruits were selected for freedom from blemish, wrapped in oiled paper to prevent storage scald, and placed on wooden trays which held from 30 to 80 fruits each, depending on the variety. These trays were stacked in such a manner as to allow free movement of air in a storage room held at 2°C.(35.6°F.). All varieties were placed in storage October 11, 1937.

Two weeks were allowed to permit the fruit to come to equilibrium at the storage temperature, and thereafter analyses were made at varying intervals. Samples of from five to eight apples were taken immediately before they were to be used and treated as described under "Determination of Ethylene in Plant Tissues."

The free-air volume of the fruits was determined as follows: A core of tissue was cut with a corkborer of 1.29 cm. diameter and sliced on a hand microtome into slices of two mm. thickness. Twenty such sections were weighed and dried in an oven at 100°C.(212°F.). The dried tissue was weighed and, taking its density as 1.55, its volume was calculated, and this volume together with the volume of the water lost in drying was subtracted from the volume of the original slices of tissue. The difference was taken as the amount of free-air space in the apple pulp.

Results

It was first determined that sodamide removed from the gases extracted from apple all substances which might reduce permanganate other than ethylene. Two hundred grams of apple tissue were sliced thin, and the gases present in the sections were removed by several short subjections to vacuum. This procedure might be expected to remove ethylene and substances but slightly soluble in water while leaving behind most of the alcohol, acetaldehyde, and esters. When apple tissue so treated was put through the regular analytical procedure, no reduction of permanganate was observed, demonstrating that the alcohol, acetaldehyde, and esters present in alcohol do not

interfere with the determination. This does not eliminate the possibility that other olefines might be present to affect the validity of the results, but the close correspondence between the results of chemical and biological analyses indicate that this is not an important factor.

Since the volume of free-air space in apples is considerable and since the ethylene present in the apple would be expected to be in equilibrium between the cell sap and free-air spaces, if there were any considerable differences in the percentage of free-air space between the different varieties, this might be related to differences in ethylene content.

When the percentages of free-air volume were determined, however, there were found to be no significantly great differences between varieties and no further regard was paid to this factor. The percentages of free-air volume found for the various species follow:

Haralson	33%	34%
Wealthy	34%	33%
Patten Greening	35%	33%
Northwestern Gr.	31%	30%
McIntosh	33%	32%
Malinda	32%	33%

Wealthy and Patten Greening

These varieties are fall apples and customarily mature on the tree. When placed in storage, the apples used in this study were plainly past their climacteric, as shown by their full flavor and softness. By December 1, their flavor was too strongly reminiscent of acetaldehyde to be pleasant, and loss of water had made the skins feel loose on the fruits. No internal breakdown was observed in these varieties or in any other varieties during the course of the experiment.

The course of ethylene content in Wealthy and Patten Greening (Fig. 4), at a high level when the fruits were placed in storage, declined rapidly, rose again, and then declined slowly and steadily to the end of the experiment. The axis on the right represents the concentration of ethylene calculated to be in the free air space of the apples at 2°C.(35.6°F.).

McIntosh

The apples of this variety when placed in storage had not reached their full flavor although the taste of acetaldehyde could be detected, indicating the approach of the climacteric. They attained full eating quality about December 1, after which decline set in. In the middle of February they were still firm and pleasant to the taste, although it was evident that their commercial storage life was about at its end.

The progress of ethylene content (Fig. 4) had reached a maxi-

mum about the time when the fruits were thoroughly ripe, after which the ethylene content declined and then rose again somewhat. Earlier work by Nelson (1937) indicates that the ethylene content falls off to about 12 gamma per cent at the end of the eighth month in storage.

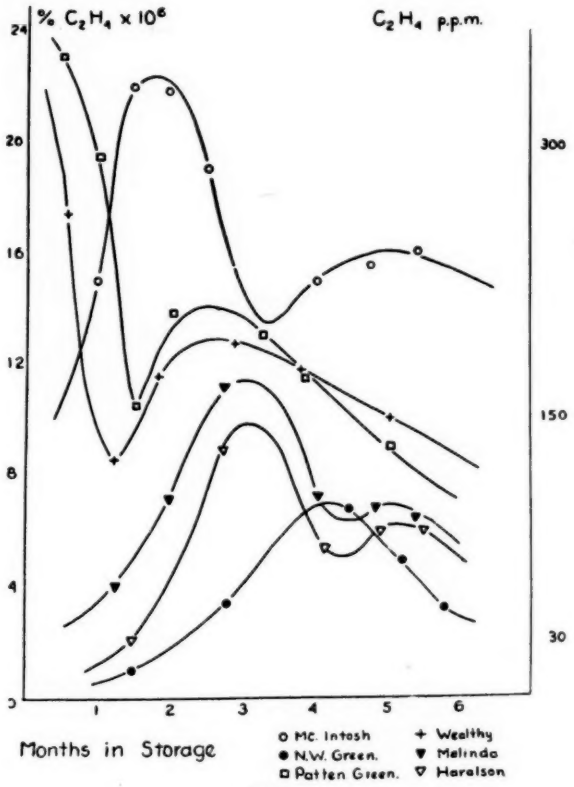

FIG. 4.

Malinda and Haralson

These varieties are of fairly good keeping quality. When placed in storage, they were still unripe. At the time of the first analysis Malinda had a slight flavor of acetaldehyde, indicating the approach of the climacteric; while Haralson was quite flavorless and lacking in sweetness. During the third month of storage Haralson became markedly sweeter and developed its characteristic flavor. These changes were accompanied by increased softness and ease of breaking down

during boiling. The changes in Malinda were somewhat less marked than those in Haralson but followed the same pattern. In these varieties the extent of decline was not great at the end of six months in storage.

The ethylene content of these varieties follows a course similar to that of McIntosh, but the maxima are much lower.

Northwestern Greening

This variety has a very long life in storage. When received, the apples were unripe and had a hard, woody texture. At the end of three months in storage there was a noticeable softening of the tissue together with a suggestion of the acetaldehyde taste. After four months the apples were completely ripe, still firm, of good flavor, and marketable at the end of the experiment.

When these apples were placed in storage the ethylene content was practically zero, and the increase to a maximum at the time of reaching ripeness was followed by a moderately rapid decline.

Discussion

From these data it may be seen that the ethylene content of all the varieties probably follows a similar course. When the apple is green, the ethylene content is low. As the apple ripens, the ethylene content increases to a maximum; and this is followed by a rapid decline, a brief rise, and a slow decline during the senescent period of the apple. Furthermore, there is a direct relationship between the earliness of reaching the maximum and the height of the maximum or, in other words, the storage life of the fruit is related inversely to its ability to produce ethylene. This may be taken as evidence that ethylene is actually a factor in the natural ripening process of the apple.

Kidd and West (1924) investigated the course of respiratory activity during the ripening process of the apple. Their findings show that respiratory activity follows a pattern somewhat similar to that followed by ethylene content, that is, respiration increases to a maximum at the height of the intensive ripening process or climacteric, falls off, then increases slightly, and finally continues its downward course.

Since the work of Harvey (1915), ethylene has been known to be a stimulant and inhibitor of respiration. Gane (1934) has shown that ethylene is not produced by the apple in the absence of oxygen. A synthesis of these facts brings forth a picture of ethylene as a regulator of the respiratory rate during the ripening process, being pro-

duced by respiration, stimulating it, and possibly inhibiting it at higher concentrations.

It may perhaps be said, therefore, that ethylene is probably connected in some way with the respiratory processes in ripening, but, lacking suitable data on the simultaneous states of ethylene content and respiratory activity, it perhaps is unsafe to say more.

MECHANISM OF ETHYLENE ACTION

Rosa (1928), working on the effect of ethylene on accelerating the ripening process in melons, concluded that the effect of ethylene was an activation of enzymatic hydrolytic processes, a view which seems to have been typical of the earlier workers in this field. With one exception, however, evidence for this mechanism is based simply on an increased velocity of formation of hydrolytic products and not on determinations of enzyme activities. The exception is the case in which Regeimbal and Harvey (1927) found that the expressed juice of ethylene-treated pineapples had a higher proteolytic activity than that of the control fruits.

This case seemed to merit further investigation, particularly since Englis and Zannis (1930) had observed no effect of ethylene on the rate of saccharification of soluble starch by taka-diastase or alfalfa meal or any effect of ethylene on invertase activity *in vitro*.

One-tenth of a gram of trypsin dissolved in five c.c. of water was added to 25 c.c. of one-per cent egg albumen solution, and the increase in free-amino groups was measured by the Van Slyke method after 24 hours. Two series of such mixtures were prepared, in one of which the enzyme and substrate were placed in an Erlenmeyer flask and corked tightly and in the other of which the air in the flask was replaced with ethylene before corking. One ethylene-treated flask and one control were incubated at each of three temperatures, 25, 35, and 45°C.(77, 95, and 113°F.). The results are shown (Table 2).

TABLE 2

Effect of Ethylene on Trypsin

Temperature	Increase in nitrogen		Turbidity of treated and control
	Treated	Control	
	mgm.	*mgm.*	
25°C.(77°F.)	1.26	0.78	.23
35°C.(95°F.)	1.53	1.02	.25
45°C.(113°F.)	1.57	1.50	.48

Evidently the treatment increased the amount of tryptic digestion which took place in 24 hours. Furthermore, it was especially notice-

able that the ethylene-treated mixtures developed far less turbidity than the untreated ones. However, it was found that if the air in the flask were replaced by hydrogen instead of ethylene, a like increase in digestion took place. An attempt to measure any adsorption of ethylene on trypsin indicated that if ethylene were adsorbed, it was only to the extent of less than .1 mg. per gram of trypsin at an ethylene pressure of 150 mm. and temperature of 20°C.(68°F.). It was concluded that the action of ethylene in this case was not specific but was simply one of protecting the trypsin from air by removal of the air from the flask.

Whether this experiment is of any value in interpreting the results of Regeimbal and Harvey is not easy to determine. Ethylene is known to be a stimulant of respiration. In a massive fruit like the pineapple there is certain to be, in any case, an oxygen deficiency in the interior of the fruit. It might be that an intensified respiratory activity occasioned by ethylene treatment would have the effect of intensifying this condition and thus, by an indirect means, protect the proteolytic enzyme of the fruit from oxygen inactivation.

In any case, it may be said that there is no known instance in which ethylene has a specific and direct effect in promoting hydrolytic processes *in vitro*.

Nord (1932), working with yeast and with zymase preparations, has ascribed a dual function to ethylene. The increase in rate of fermentation of yeast suspensions caused by ethylene treatment he ascribes to an increase in permeability of the yeast cells. The increase in rate of fermentation by zymase preparations caused by ethylene treatment he states is due to the effect of ethylene in protecting the enzyme complex from inactivation by adsorption of ethylene on the surface of the enzyme. The necessity for two explanations is not clear.

The custom of ascribing effects to changes in permeability, however, makes a measure of the magnitude of this effect desirable. Harvey (1915) investigated the effect of ethylene treatment of pea seedlings on the permeability of their cells to potassium nitrate and glycerine and concluded that neither the treated nor the untreated tissues were very permeable and that the treated ones were slightly more permeable than the untreated. Dr. J. Levitt was kind enough to determine the effect of ethylene on the permeability of the cells of cabbage petiole to thiourea and found that the effect, if any, must be very small, of the order of an increase of 15 per cent.

The writer has tested the effect of ethylene on the water permeability of potato tissue by the change-in-weight method and has

obtained similar results. The results of two typical experiments follow:

TABLE 3

Effect of Ethylene on Water Permeability of Potato Tissue

Time	Change in weight			
	I	II	I	II
min.	*pct.*	*pct.*	*pct.*	*pct.*
30	4.21	4.48	3.84	3.41
		Plus ethylene		Plus ethylene
90	3.75	4.23	3.83	3.60
150	1.61	1.68	1.34	1.42

It would hardly seem that such slight changes in permeability will suffice to account for the enormous physiological activity of ethylene.

An interesting hypothesis as to the action of ethylene has been suggested by Lynch (1936). In brief, Lynch suggests that ethylene is a "coenzyme" to the oxidizing enzymes. He places particular emphasis on the oxidase and peroxidase in fruits, and states that only those fruits which have oxidase or peroxidase activity distributed evenly throughout their tissues should respond to ethylene treatment. The reason for the emphasis on oxidase activity is not clear. As a matter of fact, the oxidase systems in banana and apple, which Lynch emphasizes, seem to have little function in the actual ripening process. Furthermore, melons in which, like all cucurbits, direct oxidase activity is absent, respond readily to ethylene treatment. Peroxidase activity cannot well be used to differentiate between tissues since it is characteristic of all living tissues.

It seems far more reasonable to suppose that the effect of ethylene is on respiratory activity rather than on oxidases. The correspondence between the course of respiratory activity and the course of ethylene content in apples tends to support this idea.

The ripening process in banana is interesting to study in this connection. At the beginning of the ripening process the respiration rises rapidly to a maximum, after which there is a gradual fall, followed by a more rapid dropping off. The onset of the rapid decline in respiration marks the end of the ripening process. The necessity for a high respiratory rate during ripening is not easy to see because the hydrolytic changes which are chiefly characteristic of it are exothermic. In order to determine what the precise relationship between respiratory activity and ethylene production might be, the following experiment was performed.

Experimental Procedure

Bananas were taken off the car green as they arrived in Minneapolis, and the fruits were cut off the hand, washed, and weighed. On a wire stand over which could be placed a bell-jar were arranged 4.35 kilograms of bananas. Within the bell-jar was placed a dish containing 100 c.c. of 5 N sodium hydroxide to absorb the carbon dioxide produced. By a connection made through the glass plate upon which the jar stood, the carbon dioxide absorbed was replaced by oxygen under a constant pressure of 1.2 cm. of mercury from a gasometer. The oxygen absorbed was calculated from a measurement of the change in level of the gasometer.

At the end of each day a 330-c.c. sample of the gas within the bell-jar was taken with a gas-sampling bulb, and the quantity of ethylene in the sample was determined in the usual manner. From this value and the known total air volume of the bell-jar the total ethylene produced by the fruits could be estimated.

The sodium hydroxide was diluted to one liter with distilled water, a 25-c.c. aliquot was taken, 50 c.c. of .4 N barium chloride were added, and the alkalinity was titrated to phenolphthalein with .396 N hydrochloric acid. The difference in titration between the original alkali and that which had absorbed carbon dioxide was a measure of the amount of carbon dioxide produced.

Results

The results obtained are shown (Fig. 5). Bananas are customarily received at Minneapolis as they approach the respiratory maximum or climacteric. The respiratory activity observed was typical. The broken line marks the end of the ripening process, the point at which all green color had disappeared from the peel; the digestion of starch was substantially complete; and the characteristic ripe flavor was prominent.

The ethylene production was relatively high over the first 24 hours, after which it declined rapidly, remained at the same low point for two days, rose briefly, and then fell off again. It will be observed that the second rise in ethylene production occurred simultaneously with the completion of the ripening process.

A potato plant was exposed to a sample of the gas removed from the bell-jar and diluted in accordance with the chemical determination to a concentration of permanganate-reducing substances calculated as ethylene, of one part in fifteen million parts of air. The potato plant gave the typical epinastic response, demonstrating that the active emanation is ethylene.

Discussion

Wolfe (1931) studied the course of starch hydrolysis during ripening in the banana and has shown that the rate of formation of sugar is at maximum at the time of the respiratory maximum and that it rises and falls off much more rapidly than the respiratory

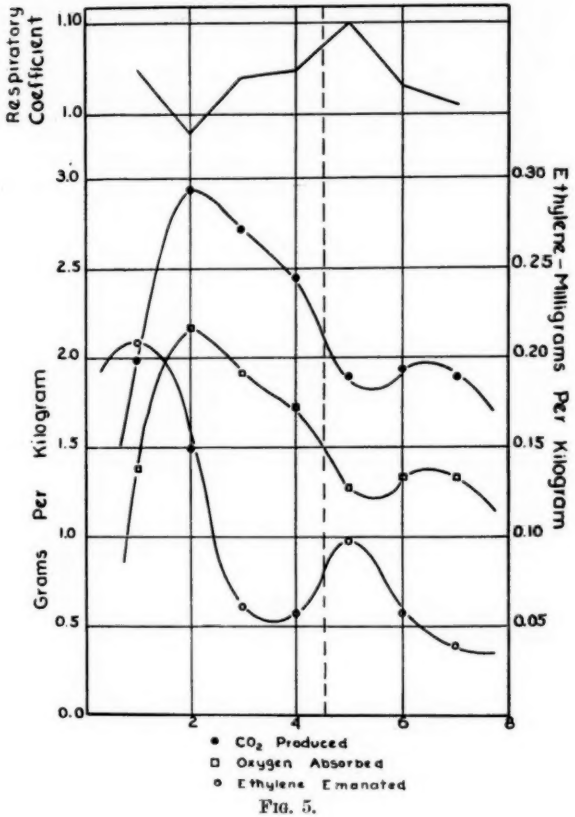

FIG. 5.

rate. He showed that although ethylene treatment seemed to have no effect on the respiratory activity of the banana, which might be expected since the climacteric is already at hand when the bananas are received, it did produce small but consistent increases in the rate of digestion of starch. He also observed that only ethylene-treated bananas lost the last trace of green at the tip, which incidentally was observed here although no ethylene was applied to the fruits.

It seems extremely probable from the evidence here presented that ethylene actually is consumed during the ripening process. How the shape of the ethylene production curve may be explained by the compounding of two simple curves representing ethylene production and consumption is shown (Fig. 6). The fact that ethylene treatment does accelerate the digestive processes although the banana is of itself producing ethylene lends weight to this probability and further suggests that the use of ethylene is connected with the hydrolytic processes in ripening.

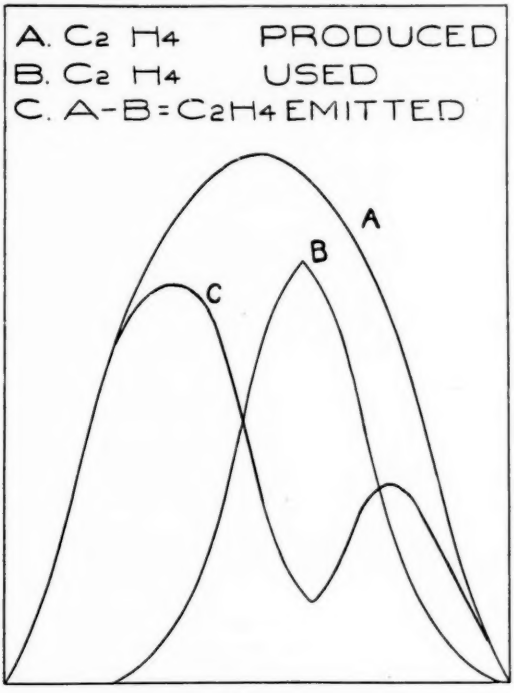

FIG. 6.

Another point of interest appears in the work of Kidd and West (1932) who state that bananas ripen as well in an atmosphere containing only 2.5 to 5 per cent oxygen as they do in air. If this be true, the respiratory processes can play no essential part in ripening, and one is forced to the conclusion that ethylene is connected with the hydrolytic processes of ripening. The idea that ethylene may also be responsible for the curve of respiratory activity does not conflict

with this hypothesis. There is no reason for believing that the respiratory activity plays any integral part in the ripening process other, perhaps, than being the means by which ethylene is produced, according to Gane (1934).

One may then conceive of ethylene as being produced by respiration and incidentally causing fluctuations in respiration to correspond to the fluctuations in the ethylene content of the tissue, while its essential function in the ripening process is concerned with the hydrolytic processes, not by any simple effect on the hydrolytic enzymes but in some manner at present unknown.

Sastri and Row (1934) found that banana amylase is inactivated by the tannin found in the banana peel. Ethylene may in some way be connected with the prevention of or protection from this inactivation. This is probably not connected with oxidative processes, however, since the work of Lloyd (1911) and of Michel-Durand (1928) indicates that the disappearance of tannin astringency from the peel is caused by the formation of a complex of tannin and a slimy carbohydrate substance.

CONCLUSIONS

A method is described for the determination of ethylene in plant tissues.

There is a general correspondence between the course of respiratory activity and that of ethylene content in stored apples.

Varieties of apples having longer storage lives are characterized by lesser abilities to produce ethylene.

Ethylene or a gas of similarly high physiological activity is produced by bananas during ripening.

Ethylene is probably consumed by bananas during the period of intensive ripening.

The fundamental function of ethylene during the ripening process seems to have to do with the hydrolytic processes, although this function is probably not any simple effect on the hydrolytic enzymes.

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BACTERIAL SPECIES ON NUT MEATS USED IN ICE CREAM MIXES

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A decided interest in the sanitary control of ice cream has been evidenced in recent years by manufacturers, state and city health department officials, and many others. In the report of the Committee on Milk and Dairy Products of the American Public Health Association Fabian (1936) states, "It is the opinion of many health officials that the time has come when more attention should be given to dairy products other than milk." He says further, "In 1929 there were only two states and one province that had laws requiring the pasteurization of the ice cream mix. Latest figures available show that now 26 states and provinces . . . require the ice cream mix or milk products used in its manufacture to be pasteurized." At the present time a number of states and cities have bacterial standards for ice cream.

While ice cream is essentially a dairy product, it does contain certain ingredients other than the milk and cream usually emphasized in sanitary regulations. Moreover, some of the ingredients, including nuts, fruits, candy, colors, and flavors, are raw products and are added to the mix after it has been pasteurized. This constitutes a potential hazard to the public health since, in addition to possible large numbers of bacteria, such raw ingredients may introduce pathogenic organisms through gross contamination in handling or storage. Since nuts may be shelled by hand under unsanitary conditions, it seemed of special interest to determine the numbers and particularly the species of bacteria present on nut meats in several ice cream plants and to observe the conditions under which they were stored and handled.

Comparatively little information is available concerning the bacterial flora of nut meats. In a report on the bacterial analysis of 314 samples of ice cream ingredients, Newman and Reynolds (1930) included one sample each of raw black walnuts, pecans, Brazil nuts, and salted peanuts and two samples of English walnuts. The samples of nuts examined were considered to have low counts, since the

[1] Based on a thesis submitted by the senior author to the Graduate School of the University of Maryland in partial fulfillment of the requirements for the degree of Master of Science.

counts obtained ranged from 120 to 2,800 per gram except with the Brazil nuts which gave a count of 46,000 per gram. Weinzirl (1929) examined seven kinds of nuts, including 600 samples, for the presence of *Escherichia coli* to determine whether or not they were a possible source of contamination in candy. *E. coli* was found on .5 to 2 per cent of the samples, a percentage slightly lower than found on candy. The author concluded, "These results do not indicate that nuts are an important source of the *E. coli* found on candy, rather, they seem to show that, like candy, they are contaminated by the hands of the vendors."

In the Report of the Chief of the Food and Drug Administration of the U. S. Department of Agriculture (1934) the following statement is made: "During the year a study has been conducted to ascertain the prevalence and significance of certain bacteria on shelled nut meats. This study is being continued to determine whether or not there is any correlation between the presence of bacteria of the colon group on shelled nut meats and the sanitary conditions under which such products are prepared for the market."

Prucha (1936) reported on the bacteria, *Bacterium coli*, and mold content of 20 samples of nut meats. Bacteria per gram varied from 0 to uncountable, molds from 0 to 470, and *B. coli* were reported in four samples. Prucha stated, "Nut meats invariably have some bacteria, particularly when the nut meats are overcooked in the process of shelling and when the nut meats are broken up in small pieces instead of full halves. When nut meats are in this condition they mold readily. The nuts are also apt to have *B. coli*. The sources of the *B. coli* are probably the hands of those shelling the nut meats."

From a sanitary viewpoint DePew (1936) suggested that mechanical slicers which come in contact with nuts should be thoroughly cleaned and sterilized, and employees who come in contact with nut meats in the plant should protect the product by using sterilized utensils and wearing rubber gloves when sorting nuts.

Sommer (1936) suggested that nut-cracking establishments be under supervision of the health inspectors since contamination may occur through humans. Tracy and Brown (1936) advised treatment of nut meats by dipping the nuts in hot [82.2°C.(180°F.)] sucrose solution for 30 to 60 seconds, followed by drying for 2.5 minutes in an oven at 121.1°C.(250°F.). They recommended subsequent storage at low temperatures preferably in glassine bags until used. With this treatment nut meats contained less than 100 bacteria per gram and were free from *E. coli*. They recommended pasteurization of other raw ingredients likely to be added to ice cream mix, such as cold-pack strawberries, flavoring extracts, and color solutions.

EXPERIMENTAL PROCEDURE

All samples examined, with the exception of a package of nut meats (Sample 1, Table 1) purchased at a local store, were obtained at nearby ice cream plants. Eighteen samples of dry nut meats were collected directly into sterile bottles. Two samples preserved in sugar syrup were transferred to sterile bottles by means of sterile wooden tongue depressors.

Accurate dilution blanks were prepared by sterilizing dilution bottles and tubes with rubber stoppers separately and filling blanks aseptically for each experiment, using tap water as diluent. Nut meats were poured from the sample container into a sterile petri dish and five grams then were transferred to tared dilution blanks by means of a sterile knife or forceps. Results were recorded as number of microörganisms per gram.

Six samples were examined in the first portion (A) of the investigation. Duplicate plates of 1-10, 1-100, and 1-1000 dilutions were poured with dextrose agar and incubated at 37°C.(98.6°F.). Duplicate plates of 1-10 and 1-100 dilutions also were poured with dextrose agar, one set was incubated at 37°C. anaerobically and another at 55°C.(131°F.) aerobically. Anaerobic conditions were obtained by using phosphorus in a jar. Duplicate plates of 1-10 and 1-100 dilutions were poured with malt agar and incubated at room temperature for one week for mold and yeast counts.

Bacterial counts were made after 24 hours' incubation if the colonies were numerous, although usually 48 hours' incubation was necessary. Colonies of the predominating types were picked and streaked on standard-methods agar slants to be used as stock cultures for further identification. Cultures were later purified by the usual procedures of plating and re-isolation. Transfers from the stock cultures were subsequently inoculated into beef broth, lactose, sucrose, and dextrose fermentation tubes, litmus milk, nitrate broth, tryptophane broth, and lead acetate agar. The cultures were identified with the aid of Bergey's *Manual of Determinative Bacteriology* (1935).

Determinations of *E. coli* were made by inoculating duplicate tubes of lactose broth with 10 c.c., 1 c.c., and .1 c.c., respectively, of the lowest dilution of the sample and incubating them at 37°C. for 48 hours. All tubes which showed gas production within 48 hours were examined by streaking Eosin Methylene Blue plates, picking typical colonies, and inoculating standard-methods agar slants following the procedure commonly used in "Standard Methods of Water Analysis" for the determination of the colon-aerogenes group. Cul-

tures containing gram-negative organisms were inoculated into Methyl Red-Voges Proskauer media and into citrate broth.

Fourteen samples were used in the second portion (B) of the investigation. Instead of using dextrose agar only, aerobic plates were poured, using both dextrose and tryptone agar, and incubated both at 32°C.(89.6°F.) and at 37°C.(98.6°F.). Also brilliant green bile lactose broth was used instead of lactose broth for the presumptive test.

RESULTS

With the first six samples (Table 1) aerobic bacterial counts on dextrose agar at 37°C. were below 2,000 per gram with one exception, i.e., the black walnuts secured at Plant B contained 60,000 per gram. Three samples showed no colonies on the anaerobic plates, the remaining three samples yielded counts of 200, 2,350, and 7,600 per gram, respectively. Bacterial counts at 55°C.(131°F.) included 274 bacteria per gram in one sample, below 100 per gram in four samples, and one sample negative. Predominating organisms isolated were gram-positive spore formers. Neither obligate anaerobes nor strict thermophiles were isolated. Colonies picked from anaerobic and 55°C. plates all grew aerobically at 37°C. Yeast and mold counts were 10, 20, and 270 per gram for three samples and negative for three samples. The presumptive test was positive for one sample, although *E. coli* was not isolated.

These results were related to some extent with preservation of nut meats and storage conditions at the plant. The lowest count obtained was with black walnuts preserved in a thick sugar syrup in a sealed can, and the highest count with raw black walnuts stored dry in a cold room. Dry storage in a cold room, while not as efficient in preserving a low count as cooking or treatment of the raw product, was apparently responsible for negative yeast and mold counts observed in Samples 5 and 6.

In the second group of samples aerobic bacterial counts on dextrose agar at 37°C. were over a larger range than in the first six samples. Eight samples gave a count of less than 1,000 per gram; three samples were below 5,000 per gram. Two samples had higher counts of 25,000 and 107,000 respectively, and in two samples plates were uncountable even in 1-1000 dilutions. With plates incubated at 32°C. four samples gave counts below 1,000, eight gave counts between 1,000 and 100,000, only one sample had a count of over 100,000 per gram, and in one other sample plates were uncountable in the highest dilution.

With tryptone agar five samples gave aerobic bacterial counts below 1,000 per gram at 37°C., four samples below 10,000, one sample

TABLE 1

Numbers and Types of Microörganisms on Nut Meats

A. Bacterial Counts on Dextrose Agar at 37°C.(98.6°F.)

Sample No.	Plant	Sample	Storage conditions	Aerobes 37°C.	An-aerobes 37°C.	Aerobes 55°C.	Gas in lactose	Yeasts and molds	Predominating types isolated
1		Black walnuts	Cellophane bag. Retail package. Room temperature.	1,562	2,350	274	—	270	*Bacillus subtilis* Actinomycete species Micrococcus species
2	A	Black walnuts	Preserved in sugar syrup in sealed cans. Retail package.	160	200	60	+	0	*Bacillus centerosporus* *Bacillus cereus* *Bacillus mycoides*
3	A	Pecans	Roasted in butter with salt. Stored in open can in warm room, partially filled with steam.	590	7,600	40	—	10	*Bacillus clostéroides* *Bacillus subtilis* *Bacillus tumescens* Actinomycete species Yeast species
4	B	Black walnuts	Small pieces in a barrel. Stored in a cold room.	60,000	0	20	—	20	*Bacillus megatherium* *Bacillus polymyxa*
5	B	Pecans	Roasted in butter with salt. Stored in a pasteboard box in a cold room.	1,200	0	0	—	0	*Bacillus cohaerens* *Bacillus mycoides* Actinomycete species
6	B	English walnuts	Stored in a pasteboard box in a cold room.	200	0	80	—	0	*Bacillus albus* *Bacillus polymyxa* *Bacillus subtilis* Actinomycete species Yeast species

TABLE 1 *(Continued)*

Numbers and Types of Microörganisms on Nut Meats

B. Bacterial Counts on Dextrose and Glucose-Tryptone Agars at 37°C. and 32°C.(89.6°F.)

Sample No.	Plant	Sample	Storage conditions	Aerobic count				Anaerobes 37°C.	Aerobes 55°C.	Gas in B.G.B. tubes	Yeast and mold count	Predominating types isolated
				Dextrose agar		Tryptone agar						
				37°C.	32°C.	37°C.	32°C.					
7	C	Black walnuts	Small pieces in a barrel at —10° C.(14°F.). New barrel opened. Sample from beneath surface.	25,000	44,500	27,400	39,300	1,430	0	+	520	*Bacillus coagulans* *Bacillus closteroides* *Bacillus polymyxa* *Bacillus subtilis* Micrococcus species
8	C	Pecans	Roasted in halves at —10°C. A new box opened.	1,000	15,000	2,620	24,850	20	0	—	10	*Bacillus albolacti* *Bacillus mycoides* *Bacillus polymyxa* *Streptococci faecalis*
9	D	Black walnuts	Preserved in sugar syrup. New can opened.	[1]	56,000	[1]	630	14,000	0	—	10	*Bacillus centerosporus* *Bacillus closteroides* *Bacillus subtilis*
10	D	Pecans	In boxes in cold room. New box opened. Not roasted.	3,500	11,000	6,300	4,700	11,200	0	+	0	*Bacillus cohaerens* *Bacillus prausnitzii* *Bacillus tumescens* *Sarcina citrea*
11	D	Pecans	Roasted and salted. In boxes in cold room. New box opened.	50	90	10	20	50	0	—	0	*Bacillus cereus* *Bacillus prausnitzii* Actinomycete species
12	E	Pistachio nuts	Ground at plant. Stored in can at room temperature. New shipment each week.	[1]	23,300	[1]	160,000	34,000	260	+	1,000	*Bacillus albolacti* *Bacillus coagulans* *Bacillus asterosporus* *Bacillus niger* *Bacillus polymyxa* *Bacillus prausnitzii* Actinomycete species

[1] Plates uncountable.

TABLE 1 *(Concluded)*

Numbers and Types of Microörganisms on Nut Meats

B. Bacterial Counts on Dextrose and Glucose-Tryptone Agars at 37°C. and 32°C. (89.6°F.)

Sample No.	Plant	Sample	Storage conditions	Aerobic count				Anaerobes 37°C.	Aerobes 55°C.	Gas in B.G.B. tubes	Yeast and mold count	Predominating types isolated
				Dextrose agar		Tryptone agar						
				37°C.	32°C.	37°C.	32°C.					
13	E	Almonds	Ground at plant. Stored in can at room temperature.	85	[1]	[1]	3,000	50	40	—	50	*Bacillus fusiformis* *Bacillus peptogenes* *Bacillus subtilis*
14	E	Pecans	Roasted in butter with salt. Stored in can at room temperature.	70	400	500	1,200	700	0	—	40	*Bacillus laterosporus* *Bacillus platychoma* *Bacillus tumescens*
15	F	Peanuts	Salted and roasted. Stored in can at room temperature.	840	2,520	1,050	1,160	0	0	—	25	*Bacillus mesentericus* *Streptococcus faecalis*
16	F	Pecans	Salted and roasted. Stored in can at room temperature.	4,200	7,100	5,600	7,700	0	0	—	0	*Bacillus subtilis* *Streptococcus faecalis* *Streptococcus ignavus*
17	F	Cashew nuts	Salted and roasted. Stored in can at room temperature.	120	260	200	140	0	0	—	200	*Flavobacterium radiatum* *Streptococcus faecalis* Leuconostoc species
18	F	English walnuts	Small pieces. Open pasteboard box at room temperature.	600	510	680	1,260	0	0	+	420	*Bacillus asterosporus* *Bacillus cereus* *Bacillus subtilis* *Lactobacillus acidophil-aerogenes*
19	G	Pecans	Roasted. Small pieces in open box at room temperature. Many dark and moldy. From previous year's crop.	1,920	4,400	430	3,450	570	0	—	6,600	*Bacillus clostercides* *Bacillus subtilis* Flavobacterium species
20	G	Black walnuts	Small pieces in open barrel at room temperature.	107,000	123,000	[1]	960,000	0	300	—	1,820	*Bacillus subtilis* *Lactobacillus helveticus* *Micrococcus subflavescens* *Sarcina citrea* *Sarcina lactea*

[1] Plates uncountable.

had over 10,000 per gram, and in four samples plates were uncountable. At 32°C. three samples gave counts below 1,000 per gram and seven between 1,000 and 100,000 per gram. There were two high counts of 960,000 and 1,600,000, respectively.

The anaerobic counts were negligible. Five samples gave negative results, five gave counts below 1,000 per gram. The highest count, 11,200 per gram, was on a sample of raw pecans. Only three samples contained organisms which grew at 55°C., the counts being 40, 260, and 300, respectively. Yeast and mold plates yielded a high count of 5,600 per gram from one sample. There were three samples negative and eight samples having counts below 1,000 per gram. Four samples gave positive presumptive tests for the coliform group but *E. coli* were not isolated.

The representative genera isolated included Bacillus, Micrococci, Sarcina, Streptococci, Flavobacterium, Lactobacillus, and Actinomycetes. The distribution of these organisms in the samples is shown (Table 2).

DISCUSSION

In the samples examined it was noted that treatment (canning in sugar syrup, roasting in butter and salt, etc.) and storage conditions (type of container, exposure to air and dust, and room temperature) influenced numbers and types of microörganisms. Treated nut meats kept in closed containers in a cold room gave relatively low bacterial counts and the flora was limited to spore-forming organisms. Those which were raw and kept at room temperature in previously opened boxes or barrels gave high bacterial counts. There also appeared to be a relationship between the size of nut meat and occurrence of a high count. Raw nut meats examined included walnuts, pistachio nuts, almonds, cashew nuts, and one sample of pecans. Two samples of black walnuts were canned in sugar syrup; pecans and peanuts had been roasted in butter and salt. Pecans and English walnuts were all in large pieces, usually in halves. The highest aerobic counts were obtained with a sample of ground pistachio nuts kept in a closed container at room temperature and a sample of black walnuts kept in an uncovered barrel.

Yeast and mold counts increased with poor storage conditions, the highest count being obtained with a sample of pecans in small pieces kept in an open box at room temperature. The box was on the floor in a dark alcove off a stairway and the nut meats were dark and obviously moldy. This plant had overestimated its output of ice cream and had bought too large a stock. The supply was then six months old but for economic reasons they felt they must use all ingredients on hand.

These results are in agreement with those obtained with ice cream by Babel (1936) and Babel and Parfitt (1936) who reported a direct relationship between yeast and mold count and total counts and indicated that yeast and mold counts might be an index to sanitary

TABLE 2

Distribution of Microbial Species in Nut Meats Sampled

Organisms isolated	Samples containing species	Total
Bacillus subtilis	1, 3, 6, 7, 9, 13, 16, 18, 19, 20	10
Actinomycete species	1, 3, 5, 6. 11, 12	6
Bacillus polymyxa	4, 6, 7, 8, 12	5
Bacillus closteroides	3, 7, 9, 19	4
Bacillus tumescens	3, 10, 14, 19	4
Streptococcus faecalis	8, 15, 16, 17	4
Bacillus cereus	2, 11, 18	3
Bacillus mycoides	2, 5, 8	3
Bacillus prausnitzii	10, 11, 12	3
Bacillus albolactis	8, 12	2
Bacillus asterosporus	12, 18	2
Bacillus centerosporus	2, 9	2
Bacillus coagulans	7, 12	2
Bacillus cohaerens	5, 10	2
Sarcina citrea	10, 20	2
Micrococcus species	1, 7	2
Yeast species	3, 6	2
Bacillus albus	6	1
Bacillus fusiformis	13	1
Bacillus laterosporus	14	1
Bacillus megatherium	4	1
Bacillus mesentericus	15	1
Bacillus niger	12	1
Bacillus peptogenes	13	1
Bacillus platychoma	14	1
Flavobacterium radiatum	17	1
Flavobacterium species	19	1
Lactobacillus helveticus	20	1
Lactobacillus acidophil-aerogenes	18	1
Micrococcus subflavescens	20	1
Sarcina lactea	20	1
Streptococcus ignavus	16	1
Leuconostoc species	17	1

conditions. Downs (1930) suggested a classification for the control of yeast and mold in strawberry ice cream which might be applied to nuts in ice cream. By this standard three samples would fall into the class of poor nut meats not to be used as an ingredient of a dairy product.

The number of bacteria per gram was found to be greater when plates were incubated at 32°C. rather than 37°C. Tryptone agar gave higher counts than dextrose agar in most instances, and the colonies were more easily distinguished and were slightly larger. These results agree with those Bradfield and Ellenberger (1936) obtained with milk, namely, an incubation temperature of 32°C. had a greater influence in producing higher counts than a modified medium. They found that combination of a modified medium and an incubation temperature of 32°C. gave highest counts especially when used for milks which had been exposed to improper cooling, utensil contamination, stable dusts, and bedding. The highest counts obtained in our work were on ground pistachio nuts and black walnuts which had been exposed to dust and air.

The presence of *E. coli* might also be used as an index of unsanitary conditions, however *E. coli* itself was not isolated from the nuts examined. Other investigators have isolated this organism from nut meats and other ingredients of ice cream and from the finished product. In this connection Yale (1936) reported upon the significance and importance of the colon test in ice cream. He states, "The colon test has much merit for the detection of recontamination after pasteurization. In many respects colon count is a fairer measure of quality than the total count."

SUMMARY AND CONCLUSIONS

Twenty samples including black walnuts, English walnuts, pecans, almonds, pistachio nuts, cashew nuts, and peanuts from six ice cream plants and one retail store were examined for total number of bacteria, yeasts and molds, and *E. coli*. Storage conditions and handling and treatment, such as roasting and preserving, apparently were principally responsible for variation in counts and types of microörganisms present.

Six samples were examined using dextrose agar incubated at 37° C., and 14 samples were examined using dextrose agar and tryptone agar with incubation at both 37°C. and 32°C. The lower temperature of incubation influenced the count and predominating species more than modification of the culture medium. Glucose tryptone agar at 32°C. consistently gave the highest counts of bacteria with the nut meats examined.

Aerobic bacterial counts varied with the different nut meats. Anaerobic and thermophilic counts were negligible. Yeast and mold counts increased with poor storage conditions. Although *E. coli* itself was not obtained, the nature of the product and methods of handling indicate that such a test should be made.

The 33 species of predominating organisms isolated were all non-pathogenic. Twenty of the species isolated were members of the genera Bacillus. One species, *B. subtilis*, was present in 50 per cent of the samples of nut meats examined.

The present study indicates that bacterial standards for nut meats would be desirable to stimulate lower counts and aid manufacturers in meeting bacterial standards for finished products.

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A STUDY OF MICROCOCCI SURVIVING IN FROZEN-PACK VEGETABLES AND THEIR ENTEROTOXIC PROPERTIES[1]

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In the course of investigations by Lochhead and Jones (1936, 1938) of organisms surviving freezing temperatures in frozen-pack vegetables, great variability in resistance to prolonged freezing displayed by different groups of bacteria was noted. It was shown that micrococci (including staphylococci) survived freezing much better than other groups so that they comprised a much larger percentage of the organisms in the frozen as compared with the fresh pack. Owing to the attention which has been focused on this group of bacteria in connection with food-poisoning outbreaks, the present investigation was undertaken to determine whether strains of micrococci isolated from frozen-pack vegetables were of the food-poisoning type, and to note whether frozen-pack vegetables provide a suitable medium for their growth and development.

STAPHYLOCOCCUS FOOD POISONING

It is only in recent years, chiefly since 1930, that certain members of the genus *Staphylococcus* have been found to be responsible for food poisoning. Staphylococcus food poisoning is generally restricted to an acute gastro-intestinal disturbance caused by the ingestion of food or drink containing toxic substances produced by growth of the organisms. It differs from other types mainly in that it is usually of a transient nature, lasting only from a few hours to several days. The onset is sudden, generally two to five hours after consumption of the affected food, and is recognized by acute abdominal pains, vomiting, and frequently purging diarrhea, sometimes accompanied by extreme prostration.

A large proportion of the outbreaks of staphylococcus food poisoning have been traced to bakery products, the majority of which were of the "cream" or custard-filled type. Milk, cheese, chicken gravy, chicken salad, butter, and tongue sandwiches have been held responsible for smaller outbreaks. Ice cream custard has been incriminated in one outbreak as reported by McCastline, Thompson, and

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Isaacs (1937). This latter case is of interest since the product, as with frozen vegetables, was packed at low temperature.

FROZEN-PACK STUDIES

In this work five vegetables were studied—asparagus, spinach, peas, beans, and corn—which had been harvested at the height of maturity. Preliminary treatment consisted of careful washing and blanching in boiling water to fix the color as well as to stop enzymatic action. After packing, the products were cooled as soon as possible in a freezer held at —17.8°C.(0°F.).

From the five products at the end of the freezing periods (1934 and 1935 packs, approximately 30 weeks; 1936 pack, approximately 16 weeks) 3,226 colonies were isolated from nutrient agar plates

TABLE 1

Micrococci in Frozen-Pack Vegetables[1]

Year of pack	Incubation temp.	Micrococci	Asparagus	Peas	Beans	Corn	Spinach
1934	20°C.(68°F.)	Colonies examined	374	293	365	337	.....
		Micrococci *(pct.)*	33.2	26.0	52.9	37.7	.....
1935	20°C.	Colonies examined	134	270	300	150	185
		Micrococci *(pct.)*	85.8	44.4	72.0	78.7	63.2
1936	4°C.(39.2°F.)	Colonies examined	12	80	24	68	.....
		Micrococci *(pct.)*	8.3	2.5	0	0	.....
1936	20°C.	Colonies examined	54	100	104	100	.....
		Micrococci *(pct.)*	11.1	58.0	23.1	36.0	.....
1936	37°C.(98.6°F.)	Colonies examined	20	80	68	108	.....
		Micrococci *(pct.)*	70.0	70.0	38.2	66.6	.....

[1] Isolations were made at end of freezing period. The 1934 and 1935 packs were frozen 30 weeks at —17.8°C.(0°F.) and the 1936 pack was frozen 16 weeks at —17.8°C.

of suitable dilution. Of this number, 1,490 or 46.3 per cent proved to be micrococci (Table 1). Nine hundred and eighty cultures were used in these experiments. Among them were many similar types, and to facilitate further experimentation it was necessary to eliminate as many cultures as possible. Cultures which appeared identical by macroscopical, microscopical, and various biochemical tests were grouped and representatives retained for further study. The number of cultures was reduced in this way to 50 different types. In addition to these, comparative tests were made with four strains of staphylococci obtained from the late Dr. E. O. Jordan, University of Chicago, and five from Dr. C. E. Dolman, Connaught Laboratories, Toronto. All cultures from Dr. Jordan were strains which had been implicated in food-poisoning outbreaks. Those from Dr. Dolman were isolated from raw milk, or from food-poisoning outbreaks, with one strain (Barss) isolated from nasal mucous membrane. All were

strains which had been found to produce enterotoxic substance according to the methods used by the above investigators.

PRODUCTION OF ENTEROTOXIC SUBSTANCE BY STAPHYLOCOCCI

Different procedures have been used to detect the enterotoxic properties of staphylococci. Barber (1914), one of the first to study this problem, isolated a staphylococcus of the *albus* type from milk. By feeding portions of milk inoculated with a pure culture of the organism to human volunteers he was able to reproduce the characteristic symptoms of poisoning noted in the original outbreak.

Jordan (1930) showed that certain strains of staphylococci were capable of generating in broth a substance which, when taken by mouth, produced a gastrointestinal disturbance. Woolpert and Dack (1933) found the enterotoxic substance to be distinct from other toxins, although they noted that whenever the enterotoxic substance was produced by a particular strain of staphylococcus, other toxins were also present and there appeared to be a rough correlation between the amount of this specific food poison and the amounts of the other toxins. In a later study Jordan and Burrows (1934) noted that food-poisoning strains lost their toxigenic power very quickly even after culturing in a semi-solid medium in an atmosphere of carbon dioxide and air, a procedure recommended by Burnet (1930) to increase the amount of toxin. They found, however, that the power to produce enterotoxic substance could be restored to a large number of cultures by transferring the organisms repeatedly on a medium containing soluble starch.

Tanner and Ramsey (1932) observed that the carbon dioxide and oxygen balance was important in toxin formation. Their results showed that non-toxic strains of *Staphylococcus aureus* were rendered toxic by changing this balance.

Kelley and Dack (1936), studying a staphylococcus isolated from tongue sandwiches, were able to reproduce typical symptoms of food poisoning by feeding artificially inoculated sandwiches to human volunteers.

Stone (1935, 1936), in an endeavor to find a test-tube method to detect food-poisoning types, developed a heavy gelatin stab medium as well as a gelatin-agar plating medium both of which were advocated for the separation of food-poisoning from non-food-poisoning types. Dolman (1934), Gwatkin (1937), Chinn (1936), and more recently Chapman, Lieb, and Curcio (1937) used Stone's media in their studies but reported them to be unreliable for detecting food-poisoning types. Chinn found that all the strains he examined, both food-poisoning and others, liquefied Stone's gelatin stab medium if

TABLE 2

Preliminary Tests on Methods of Administering Filtrates

Culture No.	Source	Treatment of filtrate	Intraperitoneal injection (3 ml.)			Oral feeding (pipette) (5 ml.)		
			Reaction			Reaction		
			Symptoms [1]	Time	Duration	Symptoms [1]	Time	Duration
				min.	*hr.*		*hr. and min.*	*hr.*
180	Dolman EA1	Boiled	D and V	35	24	None		
		Unheated				D and V	2, 40	24
181	Dolman Barss	Boiled	D effort to V	60	24	None	.....	....
		Unheated				None		
182	Dolman 2400-9	Boiled	D and V	30	24	None		
		Unheated				D	2, 25	24
183	Dolman 160-1	Boiled	None	....	....	None		
		Unheated				D	2	24
184	Dolman FP10EAn	Boiled	D	20	24	None		
		Unheated				D	2, 15	24
68	Jordan No. 1	Boiled	D and V	15	24	None		
		Unheated				D and V	4	24
69	Jordan No. 25	Boiled	D and V	30	24	None	.....	....
		Unheated				None		
70	Jordan No. 42	Boiled	D and V	20	48	None		
		Unheated				D and V	3	72
71	Jordan No. 95	Boiled	D	45	24	None		
		Unheated				D	3	24
Control	Uninoculated	Boiled (6)	D, dull, drowsy	30	24	None	.....	....
		Unheated				None		

[1] D = diarrhea; V = vomiting.

they were first subcultured on starch agar and incubated for a sufficient length of time.

DETECTION OF ENTEROTOXIC STRAINS ISOLATED FROM FROZEN-PACK VEGETABLES

The procedure adopted in this work for studying the enterotoxic properties of micrococci was similar in many respects to that advocated by Jordan and Burrows (1935) with the exception that the pH of the medium used was adjusted to 7.4. The medium was of semi-solid consistency and made up as follows:

Bacto-beef extract	3 grams
Bacto-peptone	10 grams
Soluble starch	10 grams
Shred agar	3 grams
Water	1000 ml.

In its use, 75 ml. of medium were placed in each of a series of Kolle flasks and sterilized by autoclaving for 15 minutes at 15 pounds pressure. Cultures were carried on slants of nutrient agar containing one per cent starch. Prior to use for enterotoxic studies, cultures were transferred every 24 hours for five consecutive days to insure an actively growing culture. They were then transferred for approximately six hours to a nutrient broth medium also containing one per cent starch. One-half ml. of the broth culture was used to inoculate the semi-solid medium in the flasks.

The Kolle flasks were incubated at 37°C.(98.6°F.) in a bell-jar set on a heavy glass plate and so fitted that the contents of a cylinder containing approximately 25 per cent carbon dioxide and 75 per cent air could be passed in a slow stream through the jar.

The above method gave a heavy growth on the surface of the semi-solid medium after 48 hours at 37°C. To obtain filtrates the agar was removed by passing the medium through several thicknesses of cheesecloth, then through paper, and finally through an "N" Berkefeld candle filter.

ANIMAL INOCULATION AND FEEDING EXPERIMENTS

In preliminary tests several types of laboratory animals were employed, including guinea pigs, rats, and kittens. The kitten was selected owing to the fact that it appeared to give reactions which were consistent and which simulated in many respects the symptoms observed with humans.

The method used to administer filtrates to kittens was a new one and consisted in feeding five ml. of unheated filtrate by pipette. Preliminary tests were made with this method and that recommended by Dolman, Wilson, and Cockcroft (1936). Results of these experiments are given (Table 2).

TABLE 3

Kitten-Feeding Tests With Filtrates of Micrococci (Pipette Method)

Culture No.	Micrococcus strain				Kitten reaction		
	Source	Number original isolations [1]	Stone's gelatin reaction		Symptoms [2]	Time	Duration
			Plate	Tube			
						hr.	*hr.*
44	Peas, beans	17	—	—	D	3	24
48	Corn	7	+	+	D and V	3	96
49	Asparagus, spinach, peas	10	+	+	None	....	....
50	Asparagus	1	—	—	None	....	....
51	Asparagus	1	—	—	None	....	....
53	Asparagus	12	—	—	None	....	....
55	Asparagus	5	—	—	None	....	....
56	Spinach	8	—	—	None	....	....
58	Spinach	2	—	—	None	....	....
59	Spinach	4	—	—	None	....	....
62	Asparagus, peas, beans	184	—	—	None	....	....
63	Peas	2	—	—	None	....	....
66	Beans	2	—	—	None	....	....
77	Asparagus, peas	3	⊕	+	None	....	....
78	Asparagus	1	—	—	None	....	....
85	Asparagus	2	+	+	None	....	....
86	Asparagus	1	+	+	D, effort to V	4	24
87	Asparagus	2	—	—	None	....	....
94	Asparagus, peas, beans, corn	62	—	—	D, sl. V	5	24
96	Peas, beans	10	—	—	None	....	....
98	Peas	1	⊕	+	D and V	3	24
100	Peas	1	+	+	D and V	3	24
107	Peas	1	—	—	D	4	24
108	Asparagus, spinach, peas	64	+	+	D	4	24
110	Peas	1	+	+	D and V	4	24
121	Peas	1	⊕	+	None	....	....
124	Peas	1	—	—	None	....	....
130	Asparagus, spinach, peas, beans, corn	262	—	—	D and V	4	24
137	Peas	11	+	+	None	....	....
138	Peas	3	—	—	D and V	4	24
140	Peas	1	—	—	None	....	....
144	Asparagus, peas	2	—	—	None	....	....
147	Asparagus, peas, beans	4	—	—	None	....	....
149	Peas	1	—	—	None	....	....
154	Beans	1	—	—	None	....	....
155	Beans	2	—	—	None	....	....
157	Asparagus, beans	2	—	—	None	....	....
160	Peas, beans	20	—	—	None	....	....
161	Spinach, peas, beans	108	+	+	None	....	....
162	Beans	1	—	—	None	....	....
163	Peas, beans	9	—	—	None	....	....
164	Peas, beans	4	—	—	None	....	....
166	Beans	2	—	—	None	....	....
168	Beans	4	—	—	None	....	....
170	Beans	1	—	—	None	....	....
171	Beans	1	—	—	D	4	24
172	Peas, beans	32	—	—	None	....	....
173	Beans	1	⊕	+	None	....	....
175	Beans	1	+	+	None	....	....
178	Peas, beans, corn	101	+	+	None	....	....
68	Jordan, No. 1	.....	+	+	D and V	4	24
69	Jordan, No. 25	.....	—	—	D (sl.)	3	24
70	Jordan, No. 42	.....	+	+	D and V	3	72
71	Jordan, No. 95	.....	+	+	D	3	24
180	Dolman, EA 1	.....	+	+	D, sl. V	3	24
181	Dolman, Barss	.....	—	—	None	....	....
182	Dolman, 2400-9	.....	+	+	D	3	24
183	Dolman, 160-1	.....	—	—	None	....	....
184	Dolman, FP10EAn	.....	+	+	D and V	3	24
.....	Control, uninoculated medium (14)	.....	....	....	None	....	....

[1] Grouped on basis of macroscopic, microscopic, and biochemical tests. [2] D = diarrhea; V = vomiting.

Dolman and associates (1936) made use of an observation previously reported by Jordan, Dack, and Woolpert (1931) that the enterotoxic substance was thermostable. Both groups of workers heated toxic filtrates at boiling temperature for 30 minutes, and by using their particular testing method, produced a gastrointestinal disturbance similar to that of staphylococcus food poisoning.

The principal criticism offered concerning the intraperitoneal injection method is that even filtrates obtained from sterile media were found to produce diarrhea. Six kittens, each injected with three ml. of boiled sterile medium, all showed liquid stools and were not as lively as before inoculating. They returned to normal within 12 to 24 hours after injection. Whether these reactions were due to the shock of the injection or to the presence of some irritating chemical substance in the medium is not known. The reactions noted with the sterile medium were not as pronounced as with filtrates from strains which would be designated as definitely positive. In testing Dolman's procedure for determining the presence of staphylococcal enterotoxin, Rigdon (1938) reported that in experiments with puppies and kittens the symptoms were produced by injections of the medium alone as well as by the toxin. This vitiates the reliability of results from the injection method.

It is of interest to note (Table 2) that in some instances where unheated toxic filtrates were fed to kittens typical symptoms were produced while the same filtrates, boiled for 30 minutes, caused no reaction. This would suggest that the enterotoxic substance is destroyed or greatly reduced by boiling. It is noted that with two strains (Dolman Barss and Jordan No. 25) no reaction was obtained by the pipette-feeding method. The former was isolated from nasal mucous membrane. The Jordan No. 25 strain (isolated from a food-poisoning outbreak) was found to grow poorly in comparison with the others. In a later test (Table 3) the same strain gave a slight reaction by the feeding method.

FILTRATES OF MICROCOCCI FROM FROZEN VEGETABLES

On the basis of the results obtained from the above experiment the feeding of unheated filtrates by pipette was adopted as standard procedure throughout the study. The amount of filtrate fed was approximately five ml., administered by means of a five-ml. graduated pipette. Direct feeding by pipette gave better results than the feeding of toxic filtrates in milk. Added to milk the filtrate is diluted and frequently the kitten either refuses to drink or may drink only a portion of the milk. It is believed, too, that the results obtained by the pipette method are more accurate since definite amounts may

be given. Pipette feeding is comparatively simple and kittens may be fed up to 10 ml. with minimum loss of filtrate. Mention might be made here that adult cats have been fed up to 25 ml. of filtrate and have reacted in many instances in a manner similar to that of kittens.

The typical symptoms observed with kittens, to which toxic filtrates were fed by pipette, were diarrhea and frequently vomiting. Vomiting was at times so slight that it might easily have passed unnoticed. In many instances the kittens were observed to regurgitate but did not actually vomit. Diarrhea was an outstanding symptom and the results given (Tables 2, 3, and 5) were based for the greater part on this reaction. Positive reactions generally occurred two to five hours after feeding toxic filtrates, the greatest number being noted between three and four hours.

Repeated tests with one kitten failed to indicate the development of any tolerance to the enterotoxic substance within approximately 30 days, a positive reaction being obtained each time. In all feeding experiments at least three days were allowed to enable the kitten to return to normal. None of the kittens used showed any evidence of liquid stools prior to administering filtrates.

A large percentage of the kittens obtained were infested with roundworms, a point also observed by Gwatkin (1937). Roundworms appear to have some irritant effect on the gastro-intestinal tract as noted by the appearance of feces of more liquid consistency than normal. To offset this difficulty worm capsules were administered every seven to ten days. Post-mortem examinations of the kittens showed that this treatment was effective.

RESULTS OF FEEDING TESTS

Of the 50 strains of micrococci from frozen-pack vegetables representing 980 isolations, 12 strains were positive, representing 411 cultures isolated (Table 3). All of the vegetable products tested were shown to harbor one or more strains capable of elaborating enterotoxic substance when grown in a semi-solid medium in an atmosphere of carbon dioxide and air and tested by the pipette-feeding method.

Filtrates from the four known food-poisoning strains received from Dr. Jordan were positive when fed to kittens as were also three of the five strains received from Dr. Dolman. Culture No. 69 (Jordan) grew poorly in culture, giving a weakly positive test. This same culture failed to show any definite reaction in a previous test (Table 2).

CORRELATION OF FEEDING REACTIONS WITH BIOCHEMICAL TESTS

A correlation of the results of the kitten-feeding reaction with biochemical tests is summarized (Table 4), bringing out some points of interest. It is noted that the cultures which gave positive reactions when fed to kittens were more active in fermenting carbohydrates. All cultures which gave positive reactions with kittens fermented lactose and mannitol sugar broths, a point previously demonstrated by Stritar and Jordan (1935). Of further interest is the fact that Stone's gelatin did not provide a reliable means of differentiating between cultures.

TABLE 4

Summary of Biochemical Reactions of Micrococci

Culture mediums	Positive reaction with kittens			Negative reaction with kittens		
Cultures tested (58)	20			38		
	Pos.	Neg.	Pos. pct.	Pos.	Neg.	Pos. pct.
Acid in lactose broth	20	0	100	17	21	44.7
Acid in mannite broth	20	0	100	18	20	47.4
Acid in maltose broth	18	2	90	25	13	65.8
Acid in sucrose broth	19	1	95	27	11	71.1
NO_2 in .1% KNO_3 broth	18	2	90	26	12	68.4
NO_2 in .002% KNO_2 broth	18	2	90	22	16	57.9
Litmus milk, acid	18	2	90	14	24	36.8
Litmus milk, curdling	14	4	70	10	28	26.3
Hemolysis, blood agar	12	8	60	9	29	23.7
Hemolysis, blood broth	12	8	60	9	29	23.7
Chromogenesis, blood serum	15	5	75	11	27	28.9
Liquefaction, nutrient gelatin	14	6	70	22	16	57.9
Liquefaction, Stone's gelatin	13	7	65	5	33	13.2

SUITABILITY OF VEGETABLE PRODUCTS FOR GROWTH OF FOOD-POISONING STAPHYLOCOCCI

Tests on the capacity of staphylococci of food-poisoning type to develop and produce enterotoxic substance in vegetables were made with cultures obtained from Dr. Jordan and Dr. Dolman. Peas and asparagus were used as test products, packed with two-per cent brine. Following inoculation of the vegetables, previously sterilized by autoclaving, with suspensions of food-poisoning staphylococci, initial counts were made and the material divided into two lots (1) and (2) and further subdivided (A) and (B) for different temperature storage as follows:

Lot 1 (not frozen)—

(A) held at room temperature

(B) held in electric refrigerator

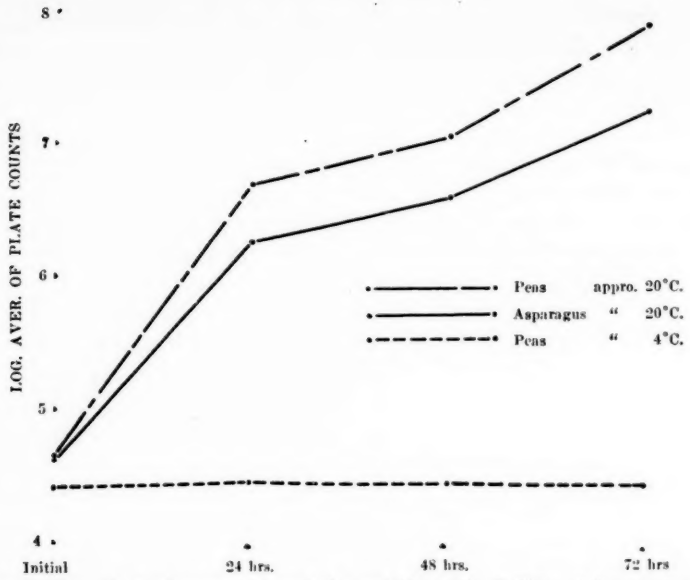

FIG. 1. Growth of micrococci in packed vegetables held at different temperatures.

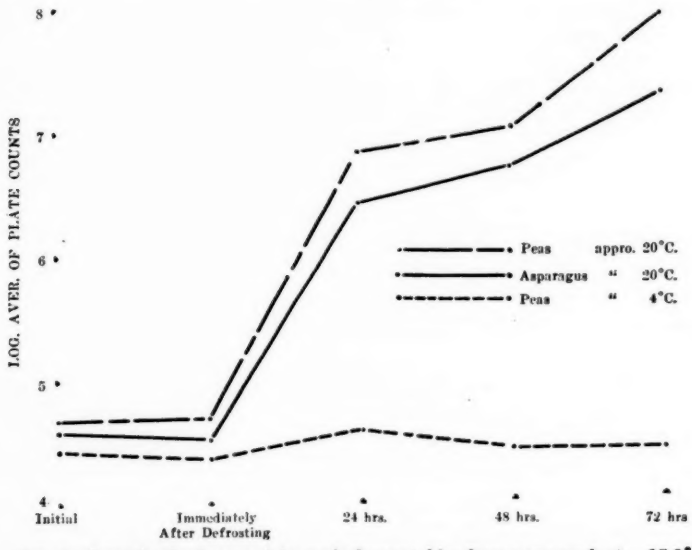

FIG. 2. Growth of micrococci in packed vegetables frozen one week at —17.8°C. (0°F.) and then held at different temperatures.

Lot 2 (held in freezer one week)—
(A) held at room temperature
(B) held in electric refrigerator.

Tests were made with eight strains at room temperature (peas and asparagus) and with five strains at refrigerator temperature (peas). Counts were made after 24, 48, and 72 hours, by plating on nutrient agar and incubating at 37°C.(98.6°F.). The results are summarized graphically (Fig. 1 and 2), showing the average (logarithmic) of all tests.

The results indicate that in both unfrozen and frozen lots staphylococci of the food-poisoning type may develop rapidly provided the products are held at room temperature. On the other hand, growth is definitely inhibited at the temperature of the electric refrigerator.

PRODUCTION OF ENTEROTOXIC SUBSTANCE IN FROZEN-PACK VEGETABLES

Since it was found that certain strains of staphylococci isolated from frozen vegetables would produce an enterotoxic substance when grown in semi-solid starch medium in an atmosphere of 25 per cent carbon dioxide, a further study was made to note whether the same organisms would form this substance in a packed vegetable. Corn was chosen, since it was the only fresh product available at the time. After inoculation (without prior sterilization) the corn was immediately placed in the freezer at —17.8°C.(0°F.). After one week at this temperature the samples were removed and divided into four lots held as follows:

(1) Room temperature, 20°C.(68°F.) for 1 day
(2) Room temperature, 20°C.(68°F.) for 3 days
(3) Household ice box, 5 to 10°C.(41 to 50°F.) for 2 weeks
(4) Electric refrigerator, 4°C.(39°F.) for 2 weeks.

Lot 2, held at room temperature for three days, had obviously spoiled and was discarded. From the other samples filtrates were obtained by the method previously described and used for kitten-feeding tests.

It was found (Table 5) that of 18 cultures tested, eight strains gave positive reactions, but only in the case of corn held at room temperature. None of the samples defrosted and held either in the ice box or in the electric refrigerator gave filtrates which produced any reaction with kittens.

EFFECT OF LOW TEMPERATURE ON ENTEROTOXIC SUBSTANCE

Staphylococci filtrates containing enterotoxic substance were held in the electric refrigerator at 4°C.(39°F.) for two months to deter-

mine whether this temperature had any effect on its potency. Results from tests with six cultures showed that the enterotoxic capacity was impaired little or none by holding at this temperature. Although no tests were made in this work to note the effect of freezing on enterotoxic substance once it has been formed by the organisms, Dack, Bowman, and Harger (1935) have shown that it resists sub-freez-

TABLE 5

Production of Enterotoxic Substance in Inoculated[1] *Frozen-Pack Corn Defrosted at Different Temperatures*

Culture No.	Source	Kitten reactions		
		Room temperature 20°C. (68°F.) 1 day	Ice refrigerator 5-10°C. (41-50°F.) 2 weeks	Electric refrigerator 4°C. (39°F.) 2 weeks
48	Corn	D, effort to V[2]	None	None
86	Asparagus	D	None	None
94	Asparagus, peas, beans, corn	None	None	None
98	Peas	None	None	None
100	Peas	None	None	None
107	Peas	None	None	None
108	Asparagus, spinach, peas	None	None	None
110	Peas	None	None	None
130	Asparagus, peas, beans, corn	D	None	None
138	Peas	D	None	None
171	Beans	None	None	None
68	Food-poisoning outbreak. Jordan	D and V	None	None
69	Food-poisoning outbreak. Jordan	None	None	None
70	Food-poisoning outbreak. Jordan	D and V	None	None
71	Food-poisoning outbreak. Jordan	D and V	None	None
180	Food-poisoning outbreak. Dolman	None	None	None
182	Food-poisoning outbreak. Dolman	None	None	None
184	Food-poisoning outbreak. Dolman	D and V	None	None
Control	Corn not inoculated (4)	None	None	None

[1] Inoculum = 5 ml. Berkefeld-filtered corn extract, administered orally by pipette.
[2] D = diarrhea; V = vomiting.

ing temperatures. The available information supports the argument against re-freezing of products once they have been defrosted.

SUMMARY

On account of the noticeably greater resistance to freezing displayed by micrococci (staphylococci) as compared with other bacteria occurring in frozen-pack vegetables, special attention was given to the relation of such products to staphylococci of the food-poisoning type.

Of 50 types of micrococci isolated from frozen-pack vegetables, 12 strains were found to produce enterotoxic substance. Determina-

tions were made by a new pipette-feeding method with filtrates of cultures grown on semi-solid starch medium in 25 per cent carbon dioxide, using kittens as test animals. The method appeared to have some advantage over other means of testing other than the use of human volunteers. All strains giving positive reactions with kittens were found to ferment lactose and mannite.

Food-poisoning strains of staphylococci may develop rapidly in vegetables held at room temperature. At approximately 4.4°C.(40°F.) no multiplication was observed. Of 18 strains capable of elaborating enterotoxic substance in pure culture in the special semi-solid medium used, eight strains were able to produce this in frozen corn defrosted at room temperature. None of the strains was able to produce enterotoxic substance in corn defrosted at approximately 4.4 to 10°C. (40 to 50°F.). The enterotoxic capacity of bacterial filtrates held for two months in a refrigerator at 4°C.(39°F.) was not noticeably affected.

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THE B VITAMINS OF CALIFORNIA GRAPE JUICES AND WINES

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This research[1] was undertaken after a search of the literature had shown only a small number of studies on the vitamin content of grapes and grape products had been made and that nothing in English had been published on vitamins in wines. A few reports on the vitamins of wine have appeared in French, Italian, and Armenian journals. Most of these do not offer quantitative data.

Daniel and Munsell (1932) and Morgan, Kimmel, Field, and Nichols (1935) reported on the vitamin content of Thompson seedless grapes, and the latter also reported on the raisins made from them. Labbé (1933) found little vitamin C and B_1 but ample B_2 in fresh grape juice and wine. Vacca (1934-35) reported the presence of small amounts of vitamin B_1 in Italian grapes, wine, and grape products. Randoin (1928, 1936) also found in French grapes and wines a little vitamin C and B_1 and a relatively large amount of the B_2 complex.

A study of the problem was undertaken in these laboratories with the object of obtaining quantitative data on juices and wines of known origin and controlled treatment. Since preliminary experiments indicated the presence of very little vitamin A in clear wines and little vitamin C in either juices or wines, the project was limited to the study of the B vitamins.

While the data reported in this paper shed considerable light on the vitamin content of certain California wines, we realize that much more research is needed in order that it may be known what effects the various cellar treatments, such as fining, filtration, etc., have on the vitamins present.

Four commercial wines, two of which were dry, Zinfandel and a Riesling type, and two fortified wines, sherry and port types,[2] were first examined for vitamin B_1 and vitamin G. The latter was deter-

[1] Assistance in part of this investigation was rendered by Works Progress Administration Official Project 665-08-3-30, Unit A-24, assigned to the University of California, and also by the California Wine Institute.

[2] These wines were supplied by the Roma Wine Company of San Francisco and this preliminary experiment was done by Helen G. Davison, whose assistance we gratefully acknowledge.

mined by the method then (1935) in use, tiki-tiki (rice-bran extract) being used as source of vitamin B_1. It appears probable from the appearance of the rats used in this assay and from the experience of other laboratories that the factor measured was riboflavin. The Zinfandel, port, and sherry wines allowed sufficient rat growth on a vitamin B_1 deficient diet as compared with that of litter-mate rats on the International clay adsorbate to indicate about eight International units of vitamin B_1 per 100 ml. of wine. The Riesling type wine appeared to have a much lower B_1 content. Similarly the three wines first mentioned allowed growth on the riboflavin-deficient diet corresponding to about 20 "Sherman-Bourquin units of vitamin G" per 100 ml., but the Riesling wine had only about one-half this value. If the riboflavin value of the Sherman-Bourquin unit is about four micrograms, as suggested by Day and Darby (1936), this would mean that the three former wines had about 80 micrograms per 100 ml., a value which agrees with those found in our later assays.

These were sufficiently positive results to warrant the undertaking of vitamin tests on experimental grape juices and wines. In the fall of 1935 four grape juices were prepared by straining the crushed grapes, packing in cans, vacuumizing and sealing, then storing at —17.8°C.(0°F.). The freezing, thawing, and careful decanting of the juices resulted in the removal of most of the tartrates, according to Marsh and Joslyn (1935). Muscat and Tokay grapes grown near Fresno and Lodi, and Zinfandel and Palomino grapes grown at Cordelia were used.

Muscat and Tokay grapes of the same deliveries as those used for the juice preparation were fermented with a starter of pure yeast to 13° and 14° Brix, then fortified by addition of high-proof brandy to 20.5 per cent alcohol. After a few days the wines were racked and flash pasteurized at 82°C.(179.6°F.). Sherry was also prepared from the Tokay wine by cooking in the usual manner at 54.4 to 60°C.(130 to 140°F.). The wines were detartrated by storing for three weeks at —17°C.(1.4°F.), thawing slowly, and decanting from the sediment. They were then filtered through pulp, bottled, and stored at —17°C. The composition follows:

Grapes	Wines	Alcohol by volume	Total acid	Volatile acid	Extract
		pct.	*gm. per 100 ml.*	*gm. per 100 ml.*	*gm. per 100 ml.*
Muscat	Muscatel	20.3	.244	.031	13.4
Tokay	Angelica	19.6	.217	.031	12.8
Tokay	Sherry	20.0	.259	.039	4.0

Dry wines were made from Zinfandel and Palomino grapes with pure-yeast starter and about 125 p.p.m. SO_2. One of the wines was

only partially detartrated. The other was frozen and thawed twice in order to remove all excess cream of tartar. There were thus two samples each of Palomino and Zinfandel wines, one considerably more depleted of tartrates than the other. All of these wines were filtered through pulp, bottled, and stored at —17°C.

VITAMIN B_1 EXPERIMENTS

Vitamin B_1 was determined first in the frozen grape juices by the rat-growth method with parallel feeding of the International clay adsorbate and sustaining supplements of a rice-bran extract. These supplements, 26 mg. daily, were given in order to allow maintenance of the animals without an unduly voluminous juice intake. The supplement was given also to the rats fed the International standard and to a group of animals without other source of vitamin B_1. As is shown (Table 1) the Muscat and Zinfandel juices were

TABLE 1

Vitamin B_1 of Frozen Grape Juices (1935 Crop) as Affected by Storage

Product	Daily dose	Number of rats	Gain in 4 weeks	Comment	International units vitamin B_1 per 100 ml.
	mg.		*gm.*		
International standard...	10	10	15	.....	.....
Rice-bran extract.....	26	16	23	.....	.....
International standard... and Rice-bran extract.....	10 26	5	45	.....	.....
Rice-bran extract..... and Palomino juice.....	26 *ml.*	....	....	.....	.....
	3.4	10	24	First assay, made	
	6.9	11	29	as soon as the juice was prepared.	4
	6.9	4	32	Assay made 6	2 - 6
	10.2	4	29	months later.	
Muscat juice.....	3.4	10	31	First assay.	10 - 19
	6.9	10	52		
	6.9	10	35	Assay 6 months	2 - 7
	10.2	6	27	later.	
Zinfandel juice.....	3.4	11	35	First assay.	10 - 14
	6.9	9	39		
	6.9	4	29	Assay 6 months	4 - 7
	10.2	5	32	later.	
Tokay juice.....	3.4	11	20	First assay.	10
	6.9	10	39		
	6.9	4	26	Assay 6 months	Trace
	10.2	6	27	later.	

found to contain measurable amounts of the vitamin when the juice was first prepared, but after six months' storage practically all vitamin B_1 value had disappeared. This loss was not unexpected since the lack of keeping quality in other respects in grape juices had long been known. Similar losses were found previously in this laboratory in the study of Thompson seedless grapes by Morgan, Kimmel, Field, and Nichols (1935). The relative lack of vitamin activity in the freshly prepared Tokay and Palomino juices may merely represent a more rapid deterioration than that shown by the Muscat and Zinfandel juices rather than a smaller original endowment.

The wines made from these juices were next examined for vitamin B_1 nearly a year after they were prepared; the results are summarized (Table 2). In this series both the International adsorbate and crystalline thiamin chloride were fed for comparison as to effect on growth of depleted young rats. Groups of six to 12 animals were used for the standards and for each of the juices and wines. One microgram of the thiamin chloride was given daily to each of the rats fed juices or wines and to the standard-fed groups as well as to some with no other source of vitamin B_1. This was done for the same purpose as the supplementing with 26 mg. tiki-tiki in the juice assay of the previous year. On one microgram daily all rats lost weight and several in each group died before the end of the 28-day test period. When 10 or 12 ml. of all the wines tested, except Zinfandel, were given along with this amount of thiamin chloride, however, none died and gains were obtained equal to those found on two to three micrograms of thiamin chloride. The wines were therefore assumed to contain nine to 12 micrograms of B_1 per 100 ml. The Zinfandel, a red dry wine, was apparently without vitamin B_1 activity.

The Zinfandel and Palomino dry wines were fed in two forms, one much less detartrated than the other, but no difference in vitamin B_1 activity was found.

Alicante grape juice of the 1936 crop was preserved in two ways, by freezing and by pasteurization at 85°C.(185°F.). Two lots of wine were made from the same juice, one fermented with a starter of Burgundy yeast, and the other with a starter of Burgundy yeast in the presence of 175 p.p.m. SO_2. Both lots were filtered through pulp and stored in quart bottles at 0°C.(32°F.). These juices and wines were assayed for vitamin B_1 one month after their preparation. Also, a second assay was made four months later on the juice preserved by pasteurization. A striking decrease in vitamin value was found in the latter case, more than half the activity having disappeared during this period of storage. This gave further confirma-

TABLE 2

Vitamin B_1 of Wines and Grape Juices as Affected by Storage and Wine-Making Procedure

Product	Reference standard	Date of assay	Period of storage	Vitamin B_1 per 100 ml.	
				Micrograms	International units
			mo.		
Wines, 1935 vintage:					
Angelica	Crystalline thiamin chloride and International standard	Nov. 1-29, 1936	12	12	4
Muscatel	"	"	12	12	4
Zinfandel	"	"	12	None	None
Zinfandel detartrated	"	"	12	None	None
Palomino	"	"	12	9	3
Palomino detartrated	"	"	12	9	3
Alicante juice, 1936 crop, frozen	"	"	1	33	9
Pasteurized	"	"	1	40	11
Pasteurized	International standard	Mar. 1-29, 1937	5	(18)	6
Alicante wine, 1936 vintage:					
Sulfited	Crystalline thiamin chloride and International standard	Nov. 1-29, 1936	1	9	3
Not sulfited			1	12	4
Muscat juice, 1937 crop, pasteurized	"	Nov. 26-Dec. 24 1937	2	32	11
Muscat wine, No. 1387	"	Dec. 22, 1937-Jan. 19, 1938	3	11	3.7
	"	Jan. 21-Feb. 18, 1938	4	10	3.0
Muscat wine, No. 1388	"	Dec. 22, 1937-Jan. 19, 1938	3	8	2.3
	"	Jan. 21-Feb. 18, 1938	4	10	3.0
Muscat wine, No. 1389	"	Dec. 22, 1937-Jan. 19, 1938	3	5	1.7
	"	Jan. 21-Feb. 18, 1938	4	3	1.0

tion of the findings reported (Table 1). The frozen juice had less vitamin B_1 even after only one month's storage than did the pasteurized juice. The Alicante wines were similar in vitamin B_1 content to the Angelica, Muscatel, and Palomino wines of the 1935 vintage but the sulfited sample had one-third less than the others.

In 1937 a large sample of Muscat juice was prepared. A portion of the unfermented juice was pasteurized in quart bottles at 85°C. (185°F.) and stored at 10°C.(50°F.). Wines made from the remainder of the juice received the following treatments:

Wine 1387 was fermented with a pure-yeast starter, filtered through pulp, bottled, and stored at 10°C.

Wine 1388 was fermented with a pure-yeast starter following the addition of about 125 p.p.m. of SO_2 to the must, filtered through pulp, bottled, and stored at 10°C.

Wine 1389 was fermented and stored similarly to Wine 1388 but clarified before bottling by fining with bentonite clay.

The Muscat juice, assayed for vitamin B_1 soon after its preparation, was found to have about the same value as the freshly prepared Alicante juice of the previous year, that is 32 micrograms of vitamin B_1 per 100 ml. A second assay of this juice was not made. The three wines were assayed twice, three and four months after their preparation. Sample 1387 had 10 or 11 micrograms per 100 ml., 1388 had 8 to 10, and 1389 had 3 to 5. Obviously only one-third of the vitamin B_1 of the juice was retained in the best of these wines, and less than this in the sulfited and clarified samples. Fining with bentonite, as might be expected, removed much of the remaining B_1, and even the usual pulp filtration in the so-called untreated wine (1387) apparently removed a large part. Sulfiting has been shown previously to be destructive to vitamin B_1.

RIBOFLAVIN EXPERIMENTS

Several of the experimental wines and the pasteurized Alicante grape juice were assayed for riboflavin on two occasions with a four months' interval. This was done by the use of crystalline thiamin chloride, as source of vitamin B_1, riboflavin-free extracts of wheat germ as source of the antidermatitis factor, which is here termed vitamin B_6, and a filtrate-factor preparation representing the unadsorbable fraction of a yeast extract in addition to cod liver oil and the basal diet. Young rats previously depleted by two to three weeks on vitamin B_2-free diet, supplemented only by cod liver oil and thiamin chloride (12 micrograms daily) were used for the feeding tests. Each of the two B_2 supplements, that is, B_6 and filtrate-factor preparations, were tested for their riboflavin content and found to contain practically none. The positive control rats were given daily

10 to 20 micrograms of pure crystalline riboflavin, the negative controls no source of riboflavin, and the others 10 ml. daily of grape juice or wine. The basal diet contained water-extracted casein,[3] 18; crisco, 3; salt mixture, as used by Hubbell, Mendel, and Wakeman (1937), 2.5; sucrose, 76.5. Cod liver oil reinforced with carotene was given separately as source of vitamins A and D.

Typical fur thinning and loss and stunting of growth was seen in the negative controls, good growth in the positives, and an interme-

TABLE 3

Riboflavin Content of Grape Juices and Wines

Product fed as source of riboflavin	Amount fed daily (micrograms)	Assay	Storage before assay	Number of rats	Gain in 28 days after depletion	Riboflavin per 100 ml. (micrograms)
			mo.		*gm.*	
Crystalline riboflavin.....	10	1	....	12	56	....
	0	....	....	12	34	....
Crystalline riboflavin.....	15	2	....	10	70	....
	0	....	....	10	½	....
	ml.					
Angelica wine.....	10	1	15	6	56	100
	10	2	19	8	58	81
Zinfandel wine.....	10	1	15	6	40	27
Palomino wine.....	10	1	15	6	44	45
	10	2	19	8	58	81
Alicante grape juice, pasteurized.....	10	2	7	8	48	23
Alicante wine, not sulfited.....	10	1	3	6	51	80
	10	2	7	8	58	81
Alicante wine, sulfited.....	10	1	3	6	51	80
	10	2	7	8	60	90

diate condition in the litter-mates on juice and wines. The Angelica, Palomino, and Alicante wines were somewhat alike in apparent riboflavin content (Table 3), 80 to 100 micrograms per 100 ml., but the Zinfandel wine and the Alicante grape juice were much lower, 27 and 23 micrograms per 100 ml. Since the wines made from the latter juice contained three to four times as much riboflavin, it must be concluded that in the seven months' storage of the pasteurized juice most of this substance had been destroyed. The low value of the Zinfandel wine in vitamin B_1 as well as riboflavin may mean that the Zinfandel juice was poorly endowed with both. No appreciable loss

[3] The washed casein was supplied by the Western Condensing Company of San Francisco by courtesy of Paul D. V. Manning and E. L. R. Stokstad.

of riboflavin occurred in any of these wines during four months' storage nor was there any obvious difference owing to the sulfiting of one of the Alicante wines.

In a similar manner the 1937 Muscat juice and wines were assayed twice for their riboflavin content. The second assay followed an interval of four months' storage in quart bottles at 10°C.(50°F.). The growth curves of the rats (Fig. 1) show that a decided destruction

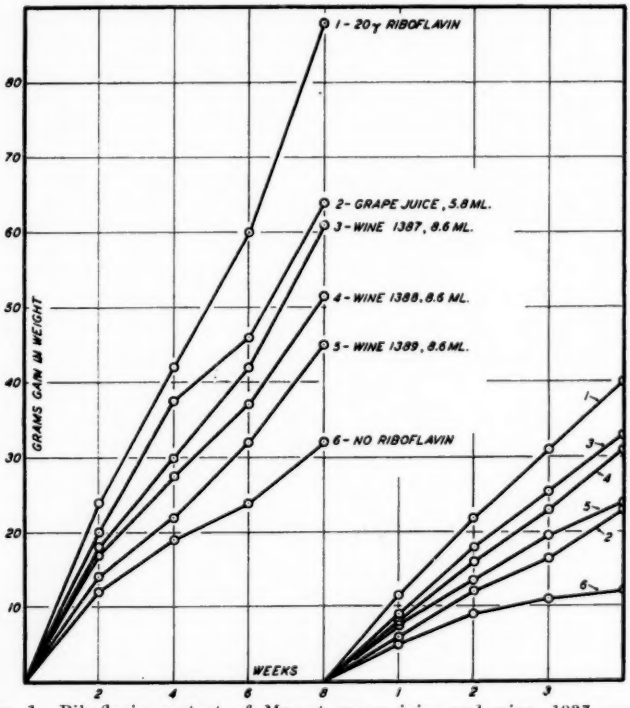

FIG. 1. Riboflavin content of Muscat grape juice and wine, 1937 crop, as affected by storage. The assay shown on the right was made four months after that shown on the left.

Wine 1387 was not treated in any way, 1388 was sulfited, 1389 was sulfited and clarified by filtering through bentonite.

of riboflavin occurred in the pasteurized grape juice, which in the first trial was calculated to contain 150 micrograms per 100 ml. and in the second 78. On the other hand the three wines maintained their riboflavin value unchanged at 140-150 for Sample 1387, 105-106 for Sample 1388, and 70-86 for Sample 1389. The four weeks' feeding period gave as clear-cut results as did the eight weeks' period.

The only comparable assay so far published is that of Kuhn, Wagner-Jauregg, and Kaltschmidt (1934) who report 8.1 to 12.5 micrograms per 100 ml. in "white wine." The method used, however, involved drastic treatment of the wine for removal of the riboflavin, its adsorption on fuller's earth, elution, and estimation of riboflavin in the eluate by colorimetric comparison with pure riboflavin. Since some of the pigment might well be destroyed or lost during these processes the results reported are undoubtedly too low. Cow's milk was reported in the same paper as containing 100 micrograms of riboflavin per 100 ml.; whereas Kramer, Gardner, Kunerth, and Riddell (1938), using a biological method somewhat like ours, found 200 to 230 micrograms per 100 ml. in cow's milk.

The best of the dry white Muscat wines made in 1937 had about two-thirds the riboflavin content of cow's milk and the bentonite-fined sample about one-third. The wines, except the Angelica made from Tokay grapes, which were reported (Table 3) as of lower riboflavin content, that is, containing one-half to one-third the amount in milk, were dry red wines. The white wines showed in nearly all cases a definite fluorescence not seen in the red wines. The riboflavin value of white wines makes it worth while probably for these wines to be bottled in dark glass and stored away from light.

VITAMIN B_6 AND FILTRATE FACTORS

Two assays were attempted in order to determine whether or not vitamin B_6 (the antidermatitis factor) and the filtrate factor or factors (greying factor in rats) are present in the wines and grape juices. In the first assay, crystalline thiamin chloride and riboflavin in adequate amounts were given all the rats so that vitamin B_6 and filtrate factors would be presumably the only, although double, dietary deficiency. A vitamin B_6 concentrate was made from wheat germ according to the method of Birch and Gyorgy (1936) and this was given in the equivalent of .5 gram of wheat germ per day to each of the positive controls as was .5 c.c. daily of a rice-bran extract containing the filtrate factors. The latter preparation was found to contain vitamin B_6 in the daily dose in equivalent of .25 gram of wheat germ but no riboflavin. The method of conducting these tests is described by Morgan, Cook, and Davison (1938).

The rats which received the wines and grape juices, 12 ml. per day in each case, had no other sources of either vitamin B_6 or filtrate factors. Those which received the Angelica and the two Alicante wines gained in the four weeks' feeding period about one-half as much as did the positive controls, while those which received Zinfandel and Palomino wines and the pasteurized Alicante grape juice

grew very little more than did the negative controls receiving no source of vitamin B_6 and filtrate factors. Several of the wine-fed rats showed some greying of the fur, thus indicating probable paucity of filtrate factor and likelihood that vitamin B_6 was the factor largely supplied in this double deficiency test. The absence of dermatitis in all wine and juice-fed rats likewise indicated the presence of vitamin B_6. The better growth of the rats given the Alicante wines than that of those given the pasteurized Alicante juice may mean either that

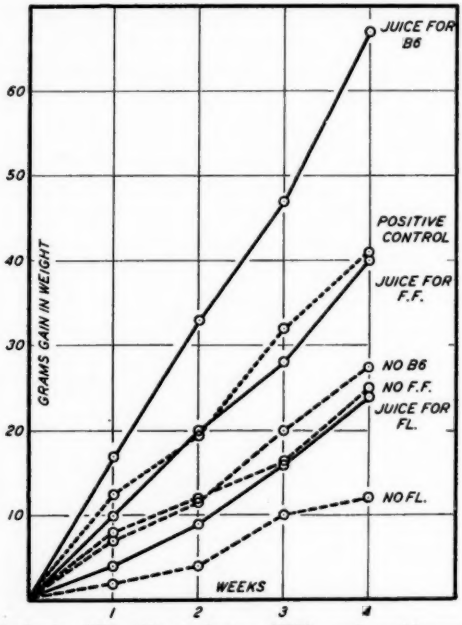

FIG. 2. Riboflavin (Fl), filtrate-factor (FF), and antidermatitis (B_6) content of Muscat grape juice (1937 crop). Ten ml. of grape juice was fed daily in each case in place of the standard dose of B_6, FF, or riboflavin.

the yeast used in vinification introduced additional B_2 factors or that these factors were destroyed on storage in the unfermented juice and preserved in the wines. The latter appears the more likely explanation in view of the similar losses of vitamin B_1 and riboflavin in stored grape juice.

In the second assay the Muscat juice was examined for its vitamin B_6 and filtrate-factor contents separately. Growth curves of the groups fed the grape juice are shown (Fig. 2). The positives were given all the necessary factors, that is, crystalline thiamin

chloride, riboflavin, vitamin B_6 as autolyzed wheat germ, and filtrate factor as the unadsorbable portion of a yeast extract.[4] These animals were entirely normal but grew at a slightly less than normal rate. The vitamin B_6 negatives had the same diet and supplements except for the B_6 preparation, the riboflavin negatives had all but riboflavin, and the filtrate-factor negatives all but the filtrate-factor extract. Three other groups similar to these three negatives were fed 10 ml. daily of the pasteurized Muscat grape juice as source of the missing factors. The group which received no vitamin B_6 had severe specific dermatitis of the kind so often described, little growth, and many deaths in the seven weeks' period. Those which received the grape juice for B_6 were normal in every respect and of larger size throughout than the positive controls. Obviously the autolyzed wheat-germ preparation did not supply the optimum amount of B_6 nor as much as did the grape juice.

The riboflavin negatives and those given the grape juice as source of this factor grew but little and showed the usual deficiency symptoms. The rats which received no filtrate factor grew less than normally, had bloody ears and whiskers, and the fur of all the grey, black, and hooded animals greyed perceptibly. The same was true of the group receiving only grape juice as source of filtrate factor, although growth in the latter was somewhat better. However, this better growth might have been due to the increased B_6 intake of this group.

The conclusion was unescapable that the Muscat grape juice contained very little filtrate factor but a large amount of vitamin B_6, and at this time very little riboflavin. Since the second assay was made seven to nine months after the grape juice was bottled it may also be concluded that the B_6 of grape juice, unlike B_1 and riboflavin, is not rapidly destroyed in storage.

EFFECT OF ALCOHOL ON VITAMIN DEFICIENCIES

In several experiments a second set of both positive and negative control animals to which 2.5 ml. of alcohol was given daily, was used in order to determine the effect of the alcohol of the wines. This was done because it had been found impracticable to dealcoholize the wines without destruction of the labile vitamins. The summary of results (Table 4) shows plainly that no failure of absorption could have occurred in the presence of the alcohol since the alcohol-fed rats grew as well as, or better than, and were in as good condition as those without alcohol when various amounts of crystalline thiamin

[4] The yeast was Strain L made by Anheuser-Busch, Inc., of St. Louis, Missouri, and supplied to us through the courtesy of G. F. Siemers.

chloride or International vitamin B_1 clay adsorbate or crystalline riboflavin were fed. Equi-caloric amounts of sugar solution were given the nonalcoholic rats instead of the alcohol solution. The vitamin B_1-deficient groups with alcohol survived seven days longer than those without alcohol and the riboflavin-deficient groups gained slightly more weight with the alcohol than without.

Both with and without filtrate-factor preparation the alcohol-fed animals compared favorably with the nonalcoholics.

TABLE 4

Effect of Alcohol on Vitamin Activity

Vitamin	Daily dose	Test period	No alcohol		With 2.5 ml. alcohol	
			Number of rats	Gain	Number of rats	Gain
Crystalline thiamin chloride	*micrograms* 0	*days* 28	8	*gm.* Dead in 18 days.	18	*gm.* Dead in 25 days.
	1	28	6	—20	6	—17
	2.5	28	7	11	6	14
International standard, B_1	*mg.* 20	28	9	54	8	57
Crystalline riboflavin	*micrograms* 0	56	11	10	11	18
	15	56	5	65	5	76
	20	56	7	98	5	92
Filtrate-factor preparation	*gm.* 1	56	5	65	5	76
	0	56	5	39	5	51

It is concluded that in the amounts fed, 10 to 12 ml. daily of a 20-per cent ethyl alcohol solution, alcohol has no injurious effect upon vitamin B_1, riboflavin, and filtrate-factor absorption or utilization in rats, and in the complete absence of these vitamins may somewhat mitigate the deficiency symptoms.

SUMMARY

Grape juices preserved by either freezing storage or pasteurization rapidly lose their vitamin B_1 and riboflavin contents.

All wines examined, red and white, dry and fortified, contained about the same amount of vitamin B_1, that is, three or four International units per 100 ml., which is one-third to one-fourth the amount present in the freshly extracted grape juices.

The vitamin B_1 and also the riboflavin of the wines were apparently not affected by aging, but were decreased nearly 50 per cent

by sulfiting of the must before fermentation and clarifying with bentonite clay during the wine-making process.

White wines were found richer in riboflavin than the red, 100 to 150 micrograms per 100 ml. in the former and 27 to 90 in the latter. All the wines tested retained this substance nearly unchanged in storage. A dry Muscat wine was found to contain 150 micrograms per 100 ml., about two-thirds the value of fresh cow's milk.

The 1937 Muscat juice was well endowed with vitamin B_6, even though the assay was made after seven months' storage. The fur of dark rats fed this juice as source of filtrate factor was decidedly greyed, and other usual symptoms indicated the absence of any large amount of filtrate factor.

Parallel feeding of vitamin B_1 standards, riboflavin and a standard filtrate-factor extract with and without ethyl alcohol (2.5 ml. daily), as well as observation of vitamin-deficient animals indicated no failure of absorption or exaggeration of deficiency symptoms owing to the alcohol.

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SPORE-FORMING ANAEROBES CAUSING SPOILAGE IN ACID CANNED FOODS

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Within the last few years a considerable number of outbreaks of spoilage in canned fruits and tomatoes in widely separated districts has been caused by a group of acid-tolerant, butyric acid-producing, spore-forming anaerobes. Products so far known to be susceptible to this type of spoilage are tomatoes, pears, ripe apricots, ripe nectarines, and figs. Juices made from these fruits have also been involved. Spiegelberg (1938) reports spoilage in canned pineapple owing to spore-forming organisms of this type. Owing to the considerable economic importance of these bacteria, representative strains have been isolated and studied.

Reported in this paper are descriptions of five strains, with extensive heat-resistance tests on one of them. The strains were isolated from the following foods:

No. 1—Spoiled canned solid-pack tomatoes
No. 2—Spoiled canned pears
No. 3—Spoiled canned solid-pack tomatoes
No. 5—Spoiled canned solid-pack tomatoes
No. 6—Spoiled canned figs in 50° Brix added syrup.

MORPHOLOGY AND CULTURAL REACTIONS

The organisms are rods with rounded ends, having a maximum length of about 5μ, and are gram positive. Spores are terminal and oval and slightly larger in diameter than the rods. Vegetative cells usually show characteristic bi-polar staining.

Sluggish motility has been demonstrated in young cultures in liver broth. All strains are obligate anaerobes.

The optimum temperature for growth is about 30°C.(86°F.). Growth will take place at 37°C.(98.6°F.) but it is slow and not vigorous. Incubation at 30°C. was used for all other tests.

Subsurface colonies in liver agar shake tubes are small and lenticular. Gelatin is not liquefied.

In litmus milk there is slow growth with reduction of litmus. No coagulation occurs and apparently there is no digestion of casein. A small amount of gas is produced, but the pH does not change.

Fermentation of Carbohydrates: Standard nutrient broth was used as the basal medium. The carbohydrates were sterilized separately in aqueous solutions for 10 minutes at 115.6°C.(240°F.) and added to the broth tubes aseptically to give a concentration of one per cent. All cultures produced considerable gas and acid (pH reduced to the range 4.1 to 5) in glucose, levulose, maltose, sucrose, trehalose, dextrin, inulin, mannitol, and sorbitol. There was a slight fermentation of l. xylose, and no fermentation of lactose, rhamnose, dulcitol, inositol, or salicin. In five other carbohydrates variations were encountered as follows:

Carbohydrate	Culture No.			
	1	2	5	6
l Arabinose	0	0	0	+
d Galactose	0	+	+	++
Melezitose	++	++	0	0
Starch	0	++	0	+
Glycerol	0	0	0	++

0 = No growth; + = Slight fermentation; ++ = Vigorous fermentation.

Volatile acid production for strain No. 1 grown in nutrient broth containing one per cent of glucose was determined by steam distillation according to the method of the A.O.A.C. 1935. NaOH was used for titration, with phenolphthalein as indicator. The following results were obtained:

	c.c. N/10 acid in 100 c.c. of culture	
	Total acid	Volatile acid
Two days' growth	38.6	21.84
Four days' growth	39.8	21.20

Judging by the odor, a considerable amount of the volatile acid was butyric.

Analyses were made of the gas produced by strain No. 1, with the following results:

	CO_2	H_2
	pct.	*pct.*
24-hour growth in liver broth	31.9	65.3
24-hour growth in tomato juice—pH 4.5	26.3	51.6

Acid tolerance was determined using vegetative cells, in canned pear juice, 10° Brix. Growth was obtained down to a pH of 3.55 with strain No. 1. In apricot juice, 22° Brix, growth has been obtained with a pH of 3.7.

HEAT RESISTANCE OF SPORES OF STRAIN NO. 1

For heat-resistance tests spore crops were obtained by growing the organism in flasks of liver broth at 30°C.(86°F.) for 16 to 20

days. The cultures were concentrated by centrifuging and stored at about 5°C.(41°F.) in a small portion of the liquid in which they had been grown.

Heat resistance was determined by the method of Bigelow and Esty (1920) using two-c.c. amounts of suspension in six-mm. inside diameter Pyrex tubes, sealed after filling. Three tubes of each suspension were removed from the water bath at each time interval and immediately cooled. The thermal death-time tubes were then subcultured into tubes of liver broth from which the air had been exhausted by boiling, stratified with vaseline to provide anaerobic conditions, and incubated at 30°C. Gas production was the criterion for growth. A one-minute lag was allowed from the time the tubes were put in the water bath until the start of the heating test was recorded. Three temperatures were used, 90, 95, and 100°C.(194, 203, and 212°F.). Foods tested were tomatoes, pears, and apricots of different pH values.

In each case (with one exception) a suspension in a neutral phosphate buffer solution was included for a check on the heat resistance of the spores.

The number of spores was determined by pouring a few c.c. of unheated suspension into a test tube, placing the cold tube in boiling water for two minutes, cooling, and making a count by dilution in liver broth tubes.

The detailed results of the heat-resistance tests are shown (Table 1). Values obtained from the thermal death-time curve in combination with heat-penetration data are shown in the calculation of processes for canned foods according to the method worked out by Ball (1928). The significance of these values is discussed at some length by Townsend, Esty, and Baselt (1928) in a recent publication. The manner in which a thermal death-time curve is drawn is shown (Fig. 1).

SOURCE AND DISTRIBUTION

In one cannery in which extensive spoilage had been encountered in tomatoes and figs a bacteriological survey was made of all equipment with which the tomatoes and figs might have come in contact and which might have served as a source of contamination if the organisms became seeded there and multiplied. No evidence of contamination was found, and it was concluded that the spoilage bacteria were brought in with the tomatoes and figs from the fields and were not all removed by the washing and, in the case of tomatoes, by the peeling and coring operations. To check this possibility, samples of soil were obtained from a number of tomato fields and fig groves, thoroughly dried and sieved, and used to inoculate experi-

TABLE 1

Heat Resistance of Spores of Strain No. 1 in Various Media

Run No.	Suspension No.	Number of spores per tube	90°C. (194°F.)		95°C. (203°F.)		100°C. (212°F.)	
			Survival	Destruction	Survival	Destruction	Survival	Destruction
			Phosphate Buffer—pH 7.0					
			min.	*min.*	*min.*	*min.*	*min.*	*min.*
1	1	Not determined	.....	.....	.....	.....	30	40
3	3	100,000	180	No end point	.....	.....	....	....
4	3	20,000	360	420	120	No end point	30	40
5	3	20,000	420	480	120	150	25	30
6	5	2,000	180	240	.....	.....	....	....
7	5	20,000	360	420	120	No end point	28	No end point
8	5	20,000	300	370	120	150	20	25
9	6	2,000	300	370	120	150	25	32.5
			Tomato Juice—pH 4.5					
2	2	Not determined	.....	.....	.....	.....	15	20
3	3	100,000	180	No end point	60	75	6	10
4	3	20,000	210	240	75	90	8	10
8	5	20,000	120	150	45	60	6	8
			Tomato Juice—pH 4.1					
3	3	100,000	50	No end point	.....	.....	5	7
4	3	20,000	120	150	30	40	9	12
8	5	20,000	135	No end point	20	27	2	4
			Tomato Juice—pH 3.8					
8	5	20,000	80	100	15	20	4	6
			Canned Pear Juice—10° Brix, pH 4.5					
5	3	20,000	150	180	45	60	15	20
			Canned Pear Juice—10° Brix, pH 4.15					
5	3	20,000	120	150	40	50	10	12
			Canned Pear Juice—25° Brix, pH 4.5					
5	3	20,000	120	150	45	60	12	15
			Canned Apricot Juice—22° Brix, pH 4.5					
7	5	20,000	120	150	35	45	7	10
9	6	20,000	40	60	30	40	4	6
			Canned Apricot Juice—22° Brix, pH 4.15					
7	5	20,000	45	60	15	20	4	7
9	6	20,000	22	30	No growth	11	2	4
			Canned Apricot Juice—22° Brix, pH 3.95					
7	5	20,000	30	45	15	20	4	7
9	6	20,000	22	30	7	11	2	4

mental lots of solid-pack tomatoes in No. 1 tall cans (dimensions $3\frac{1}{16}$ in. diameter and $4\frac{11}{16}$ in. height). The tomatoes used in this pack were all from the same ranch. In canning, each tin was half filled, inoculated with a quarter of a standard teaspoonful of soil (about .6 gm.), and then filled. The cans were exhausted 12 minutes at 97°C.(206°F.), cooked 20 minutes at 100°C.(212°F.) in a water bath, and water cooled. The center can temperatures at the end of

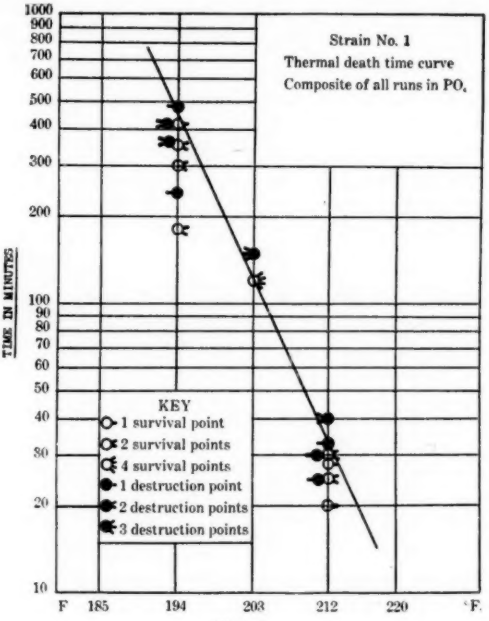

FIG. 1

the cook varied from 79 to 82.2°C.(174 to 180°F.), with an average of 81°C.(178°F.). The cans were incubated for seven weeks at 30°C. (86°F.) and spoilage was determined by the bulging of the ends owing to gas production; the results of this experiment are shown (Table 2).

Spoilage was found to be due in all cases to butyric acid-producing rods. They were evidently widely distributed in the soil of the ranches investigated.

DISCUSSION

The strains of *Clostridia* described in this paper appear to be closely related to *Clostridium pasteurianum*, Winogradsky (1895, 1902) and Omeliansky (1916).

These bacteria are widely distributed in the soil, and owing to their high sugar and acid tolerance, and the comparatively high resistance to heat of their spores in acid media, are of considerable importance to the canning industry. They grow readily in suitable media with a pH above 4, and more slowly in a pH of 3.6 to 4. As examples of the signficance of this characteristic, in the annual canned fruit and vegetable cuttings of the National Canners Association, Western Branch Laboratories (1932-37), pII values of solid-pack tomatoes for 1934 to 1937 inclusive have been found to vary

TABLE 2

Spoilage in Experimental Pack of Tomatoes Inoculated With Soil

Soil sample	Product grown on soil	Number of cans	Number of swells	Percentage of swells
Uninoculated control cans	.....	24	1	4.2
No. 1	Tomatoes	24	10	41.7
No. 2	Tomatoes	24	4	16.7
No. 3—Heavy loam	Tomatoes	24	11	45.9
Sandy loam	Tomatoes	24	11	45.9
No. 4	Tomatoes	24	8	33.3
No. 5—Ranch 1	Tomatoes	24	6	25.0
Ranch 2	Tomatoes	24	17	70.9
Ranch 2	Beets	24	22	91.7
No. 6	Figs	24	6	25.0
No. 7	Figs	24	6	25.0
No. 8	Figs	24	12	50.0

from 4.1 to 4.6, with the average in the upper part of the range; Bartlett pears from 3.6 to 4.5, with the average about 4.1; and ripe Blenheim apricots from 3.4 to 4.3, the average in this case being below 4. The pH of canned figs varies from 4.5 to 4.9. It is evident from these figures that these products are all susceptible to spoilage from the bacteria described here.

The problem of control is a difficult one, as spoilage does not appear to be the result of factory contamination (at least with figs and tomatoes) but of direct inoculation of the fruit and tomatoes from the soil. There is evidence that water may sometimes be a carrier of these bacteria. Soil analyses can be made to determine the number of significant spoilage types present, and fruit from areas found to be highly contaminated could be set aside and not used for canning. However, in many cases this would not be feasible. Thorough washing and even peeling of the fruit is apparently not an effective means of control, although the spoilage hazard is undoubtedly reduced. There remains acidification of the fruit for canning, as reduction in the pH has a marked effect on the resistance

to heat of bacterial spores (Table 2). The data indicate that to destroy spores of these organisms in tomato juice at 100°C. takes twice as long at pH 4.5 as at pH 4.1, and nearly five times as long as at pH 3.8. Figures for pears and apricots are not quite so striking, but only one or two runs were made with these products, an insufficient number for proper time-temperature relationships. The addition of acid to canned fruits, fruit juices, and tomatoes, therefore, would markedly reduce the time necessary for sterilization. The only alternative is a very severe process, which causes considerable damage to the food product.

SUMMARY

A group of butyric acid-producing, spore-forming anaerobes is described. The organisms have a high tolerance for acid and sugar and are closely allied in their characteristics to *Cl. pasteurianum* Winogradsky. They are of considerable commercial importance in the spoilage of low-acid canned fruits and tomatoes, as their spores have a comparatively high resistance to heat in acid media. Contamination seems to occur in the field and not in the cannery, and the organisms appear to be present in the soil of certain districts in large numbers.

Possible control measures are discussed. Acidification is suggested as the most feasible.

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AN ACTINOMYCES-LIKE ORGANISM ASSOCIATED WITH A FOOD-POISONING OUTBREAK

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It is a familiar fact that certain bacteria are concerned in food poisoning. Some members of the Salmonella group and poisonous substances produced in food by *Clostridium botulinum* and *Staphylococci* are the most frequent and important causes of food poisoning. That other bacteria may be involved has often been suspected. *Proteus, Streptococci, Serratia marcescens, Bacillus subtilis* and *Bacillus coli* have been incriminated, Tanner (1933). It might also be mentioned that several outbreaks of shell-fish poisoning due to a plankton, *Gonyaulax*, have been recorded by Sommer and his coworkers (1937).

The outbreak reported here may be of interest because the organism apparently involved was one which, so far as is known, has never been incriminated in food poisoning.

In January, 1938, two cases of acute gastroenteritis were observed by a physician in Charleston, South Carolina. Typical food-poisoning symptoms were observed with diarrhea, vomiting, cramps, and prostration. The symptoms were severe for about a week but soon after became mild, and recovery was complete. An interview disclosed that the cases, two middle-aged women living together, had partaken of a breakfast of cooked hominy, canned fish roe, corn muffins, and coffee. The symptoms appeared in both women about one hour after the meal. They later stated the fish roe had tasted unusual and "burned their mouths." The can of fish roe was first opened at the time of the meal.

Specimens of the hominy, fish roe, and corn muffins were obtained by the physician and brought to the laboratory on the day of the outbreak. They were kept in the ice box over night and examined bacteriologically the following morning. The specimens of food were streaked on nutrient agar plates, eosine-methylene-blue plates, and inoculated into veal-infusion broth. Anaerobic cultures were also made. No growth was obtained from the hominy and a few colonies of gram-positive, aerobic sporeformers were found on the plates streaked with small pieces of the muffins. The plates streaked with the fish roe contained numerous colonies along the line of streak and around each particle of fish roe which had been deposited on the

plate at the time of streaking. All of these colonies were of the same type.

A chemical examination of the specimens of food by another laboratory disclosed no evidence of the presence of the more common chemical poisons. The bacteriological data, however, suggested that the etiological agent might have been introduced by means of the fish roe.

STUDY OF ISOLATED ORGANISM

A study was made of the organism isolated from the fish roe. Because of the unusual nature of this organism it is believed a detailed account of its characteristics may be of interest.

Morphology: On culture media the morphology is variable. There are several factors influencing this variation—the temperature of incubation, the age of the culture, and the medium. Greater variation is seen in cultures incubated at 37°C.(98.6°F.) than at 22°C. (71.6°F.). At the latter temperature both young and old cultures are always gram-negative, short rods. On various media and in cultures incubated for 1 to 14 days at 22°C. the length of the cell varies from 2μ to 5μ (Figs. 1 and 2). In liquid and some solid media chains of cells are formed (Fig. 2). The organism is motile at 22°C. but is not motile when incubated at 37°C.

At 37°C. the organism is exceedingly pleomorphic. Great variation in morphology occurs as the culture ages at this temperature. In young cultures the cells are gram negative but some forms, which appear in older cultures, contain gram-positive granules inside the cells. If a 24-hour culture, which has been incubated at 22°C. (its optimum temperature), is streaked on an agar slant (or other solid medium, such as carrot, potato, Ca-malate agar, Czapek's agar, veal-infusion agar, Loeffler's medium, or blood agar) and incubated at 37°C. the morphological changes can be observed if smears are made daily for two weeks.

In 24 hours at 37°C. a number of long, darkly stained rods appear, although the predominant cell is still the short rod. After two to four days there are fewer short rods; the long rods become more numerous and long filaments begin to appear. In five to eight days the filaments increase in number, and deeply stained granules can be seen in the cells. Budding forms are also present at this time on some media (Fig. 9). Club forms and irregular forms are found in 10 to 12 days as well as "ghost cells" containing chains of deeply stained coccoid bodies (Fig. 12). After this period the irregular forms increase in number and many extra-cellular coccoid bodies are found. The cultures still contained viable cells after two to three weeks at 37°C. as transfers grew out at 22 and 37°C. At no time

were branching forms encountered either at 22 or 37°C. on any of the media used. More bizarre types developed on carrot, potato, and Loeffler's medium.

If an agar-slant transfer from a 48-hour, 37°-C. culture is incubated at 22°C. and examined periodically, every four hours during the first day and then daily for about a week, the filaments can be seen to break up (segmentation) into long and then short rods.

Cultural Reactions: Growth is readily obtained on the usual laboratory media and is most abundant on nutrient agar and nutrient broth at 22°C. Under reduced oxygen tension growth is poor. On synthetic media growth is very poor at 37°C. but somewhat better at 22°C.

On an agar plate colonies are round, smooth, raised, discrete, and non-pigmented. In 24 to 48 hours they reach a size of two to three millimeters. No radiating filaments are seen surrounding the colonies in cultures incubated at 22 or 37°C. and the margin of the colony is regular. In liquid media a granular growth develops which settles to the bottom of the tube. Pellicles, which form successively, also settle.

Biochemical Characteristics: Glucose and xylose are fermented with the production of a small amount of acid at the surface of the broth in 48 hours at 22°C. On further incubation the acid is neutralized and in 10 days the indicator is reduced. In lactose, sucrose, rhamnose, mannite, galactose, inulin, arabinose, trehalose, levulose, salicine, and inosite no acid is produced.

Nitrate is reduced to nitrite, cellulose is not digested, and starch is not hydrolyzed. The tyrosinase reaction is positive. Milk is coagulated (rennin curd) in 12 days at 22°C. Gelatine is liquefied in 48 hours. No pigment is produced.

Resistance to Heat: All cultures tested, regardless of age, are killed in 30 minutes at 65°C.(149°F.).

Animal Experiments: Rabbits and guinea pigs were inoculated intravenously and fed by mouth with living and dead cultures. Cats were fed sterile filtrates of broth cultures. Rabbits lost from 10 to 15 per cent of their original weight in 24 to 72 hours after inoculation or feeding. Diarrhea was noted in one animal for several days after inoculation with a killed culture. One rabbit died 18 hours after inoculation with a killed culture, while another died eight days after inoculation with a living 22°-C. culture. Most of the rabbits which lost weight during the first few days later regained the weight lost and appeared normal. No reaction was noted in rabbits fed with sterile filtrates. Guinea pigs also lost considerable weight but no deaths occurred. Cats fed with cultures or with sterile filtrates

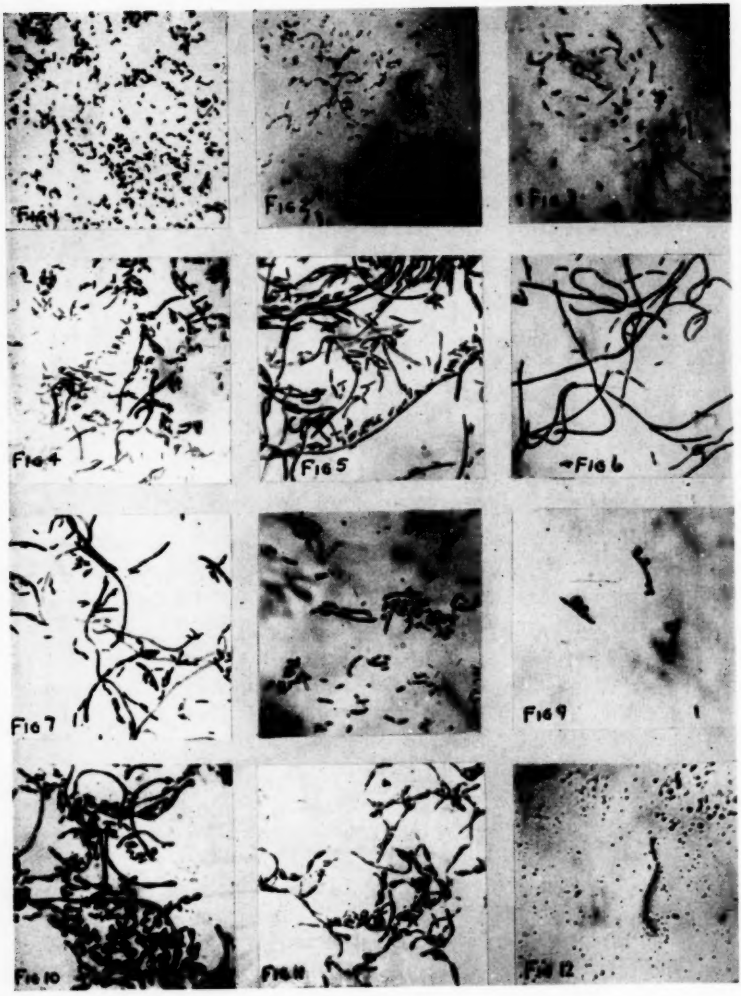
Fig 1
Fig 2
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Fig 11
Fig 12

PHOTOMICROGRAPHS

FIG.

1. A 24-hour culture on nutrient agar at 22°C.(71.6°F.). Short, gram-negative rods.

2. A 24-hour culture on Ca-malate agar at 22°C. Gram-negative rods and chains of rods.

3. A 24-hour culture on Ca-malate agar at 37°C.(98.6°F.). Gram-negative rods with gram-positive granules.

4. A 24-hour culture on nutrient agar at 37°C. Long and short gram-negative rods.

5. A 48-hour culture on nutrient agar at 37°C. Filaments and long and short rods, all gram negative. In the center of the field a few faintly stained filaments with darker stained bands can be seen.

6. A 48-hour culture on nutrient agar at 37°C. Swollen cells and banded filaments are seen.

7. A five-day culture on nutrient agar at 37°C. Filaments breaking up into short rods. Faintly stained band forms also present.

8. A six-day culture on Czapek's glycerol-dextrose agar, 37°C. Rods containing one or more granules.

9. A five-day culture in dextrose broth, 37°C. Buds (or spirals?) projecting from cells.

10. A six-day culture on carrot slant at 37°C. Filaments, club, and dumb-bell forms.

11. A 12-day culture on carrot slant at 37°C. Club forms and swollen cells as well as short and long rods are seen. The clubs in this and in Fig. 10 are gram positive. The rest of the cell is gram negative.

12. A nine-day culture in nutrient broth at 37°C. Chain of granules in a faintly stained sheath (ghost cell). This is a filament probably breaking up into granules many of which can be seen in the field. These granules are gram positive, while the faintly staining sheath is gram negative.

did not react during a five-hour period following the feeding nor was any effect noted during five succeeding days.

Classification: The exact identification of this organism has not been determined. From the morphological point of view the organism has characteristics similar to the Actinomyces but there are apparent differences (absence of branching) which prevent classification into this group. It also possesses similarities to some of the soil members of the Coryne-bacterium group and to the Proactinomyces to which it may be related. The pleomorphic nature of the organism places it very broadly among the Actinomycetales and perhaps closer to the Proactinomyces, a group of soil organisms described by Topping (1937).

Etiological Significance: The presence of large numbers of the Actinomyces-like organism in the fish roe, to the exclusion of other bacteria and metallic poisons, points with suspicion to this organism as the cause of the food poisoning. The unusual flavor observed by those involved in the outbreak also points to the fish roe as the possible agent. The other foods examined apparently played no part in this case. The samples of food were secured within a very short time after the food was consumed, collected in sterile containers, and kept in the ice box until they were examined. The presence of the organism in such large numbers indicates that they must have been present at the time the food was consumed. How the contamination occurred could not be determined but it appears that the can of fish roe was contaminated before it was opened and that the organism had somehow survived the canning process. Several cans of the same brand of fish roe were purchased and on examination were found to be sterile. The organism isolated was, therefore, the only evidence found for the cause of the food poisoning.

SUMMARY

An outbreak of food poisoning involving two people is recorded. The food involved was apparently fish roe contaminated with a pleomorphic Actinomyces-like organism. Because of the unusual nature of this organism its characteristics are described in detail.

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PRECOOLING OF POULTRY

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The preparation of market poultry involves bleeding, plucking, and cooling the product to a temperature near the freezing point, before the birds are graded and packed for subsequent storage or transport. For obvious reasons this initial cooling, commonly termed precooling, should be accomplished quickly. The present study was undertaken to provide quantitative information on the time required for precooling. Other factors, such as shrinkage; retention of bloom during subsequent storage in the frozen state; and bacterial content of the water used for precooling, where this method was employed, were also investigated in a preliminary way. The effect of various cooling and freezing practices on other qualities of the product have been discussed in an earlier paper by Sair and Cook (1938).

The time required to cool the product to a given temperature depends on the properties of both the cooling medium and the product. The properties of the cooling medium—whether liquid or gas, its temperature, rate of circulation, etc.—need not be considered here, since only a limited range of these conditions is available or possible for precooling purposes and these are comparatively well understood. Any property of the product affecting its heat capacity or conductivity will also affect the precooling time. Such factors as the initial temperature and weight of bird can be estimated quantitatively and related to the time required for cooling. Other factors, such as the shape of the bird, which affects the surface-to-volume ratio; and the content and distribution of moisture and fat in the carcass, discussed by Stiles (1922), will also affect the heat capacity and conductivity, and such quantities cannot be measured with sufficient precision to determine their influence on the rate of cooling. By measuring the initial temperature, weight, and precooling time of a sufficient number of birds chosen at random, it was possible, by statistical analyses, not only to relate these three observed quantities but also to gain some idea of the relative influence of the unknown quantities from the residual variance.

[1] Issued as paper No. 8 of the Canadian Committee on the Storage and Transport of Food.

PRECOOLING TIME

The birds were killed, dry rough-plucked, wax-dressed, and removed to the precooling room at once. All temperatures were taken immediately after weighing and at one-hour intervals thereafter by means of thermocouples connected to a recording instrument. In certain preliminary experiments on cooling rates in air, two thermocouples were used for each bird. One of these was placed just under the skin on the breast to determine the surface temperature and the other in the center of the bird to determine the internal temperature. In the main series of tests the internal temperature only was measured.

The air temperature in the precooling room was 0°C.(32°F.) throughout. The relative humidity was about 70 per cent initially and increased to about 75 per cent for a short time after the birds were placed in the space and then returned to the initial value. An intermittent air circulation, under the control of a thermostat, at a mean rate equivalent to about ten changes per hour was used throughout. These conditions are probably somewhat drier than those used in commercial practice.

The birds cooled in water were immersed in tanks placed in the precooling room. The amount of water used was always in excess of ten times the weight of product immersed at any one time. Under these conditions the water temperatures changed slowly and enabled the thermostatic devices to maintain the required temperatures of 0 and 7.2°C.(32 and 45°F.). The birds were placed in the tanks as soon as received and removed two hours later regardless of their internal temperature. They were then allowed to hang in the room at 32°F. to dry and finish cooling. Hourly temperature records were taken throughout the cooling period whether the birds were in water or air; and all computations were based on the time required to reach the desired internal product temperature, whether it was reached in the water or subsequently while hanging in air.

As the final stage of cooling was accomplished in a medium at 32°F. in all experiments, an internal temperature of 1.1°C.(34°F.), i.e., two degrees (Fahrenheit) higher, was taken as the end point. The time required for cooling was taken from the time the product was received at the cooling room until it reached 34°F., and was subsequently read from the chart on the recorder, interpolations being made where necessary.

The results of preliminary experiments, in which the weight and other factors were standardized as far as possible, showed that a linear relation existed between the logarithm of the product temperature and the time in the precooling room. Since the rate of

cooling is determined by the temperature gradient between the product and the cooling medium, and since air temperatures either above or below 32°F. may be used in practice, it was felt that the results would be more generally applicable if the temperature gradient rather than the product temperature was used in subsequent computations.

Typical curves obtained from the results of preliminary experiments (Fig. 1) show that the temperature gradients between the center of the product and the air; the skin and the air; and the

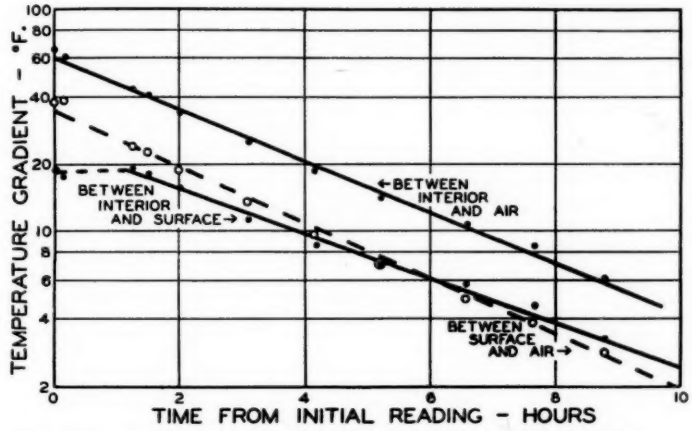

FIG. 1. Relation between logarithm of temperature gradients and time.

center and the skin, once a steady condition has been attained, are all linear functions of time within the variability ordinarily encountered. The temperature gradient between the interior of the product and the air is naturally the largest, being the sum of the other two gradients measured. It is evident that the gradient between the surface of the product and the air falls off more rapidly with time than the gradient between the product interior and the air. The reverse is naturally true of the gradient between the interior and surface of the product. The two lower curves (Fig. 1) cross six hours after the experiment was started, or generally speaking, the two gradients become equal at about half the total time required for cooling. The behavior of these two curves indicates that transfer of heat from the surface of the product to the air determines the rate of cooling during the first half of the period, while transfer of heat from the interior to the surface determines the rate during the second half. Under these conditions it would appear

TABLE 1

Regression Equations Relating the Cooling Time of Poultry to Initial Temperature and Weight

Description	Degrees freedom	Equation	No.
Cooled in air at 0°C.(32°F.)—			
Linear. Type equation [1]	....	$T = K + b_1 \log (t_p - t_m) + b_2 Wt.$	
First experiment only	27	$T = -7.7 + 6.84 \log (t_p - t_m) + 0.50 Wt.$	(1)
First and second experiments	56	$T = -5.0 + 6.23 \log (t_p - t_m) + 1.16 Wt.$	(2)
Second degree. Type equation	....	$T = K + b_1 \log (t_p - t_m) + b_2 Wt. + b_3 Wt.^2$	
First experiment only	26	$T = -4.7 + 6.61 \log (t_p - t_m) - 1.36 Wt. + 2.76 Wt.^2$	(3)
First and second experiments	55	$T = +2.8 + 6.12 \log (t_p - t_m) + 1.23 Wt. - 0.09 Wt.^2$	(4)
Cooled in water at 32°F. for 2 hours then in air at 32°F.—			
Linear	8	$T = -0.53 + 1.26 \log (t_p - t_m) + 0.38 Wt.$	(5)

[1] T = Time in hours to pass from initial temperature to temperature of cooling medium + 2°F., i.e., 34°F. in these tests. K = Initial constant. b_1 = Regression coefficient, temperature gradient. t_p = Initial product temperature. t_m = Temperature of cooling medium. b_2 = Regression coefficient, weight. b_3 = Regression coefficient, (weight).^{2} Wt. = Weight in pounds.

that immersion in water for a short period after dressing would be of material assistance in reducing the time required for cooling.

In the main body of experiments it appeared preferable to omit measurements of the surface temperature of the product in favor of making more measurements on the internal temperatures of the birds. With the equipment available this enabled twice as many birds to be studied at a given time. Only one thermocouple was placed in each bird but an estimate of the standard error of the temperature gradient (i.e., a difference) indicated that it was less than one degree Fahrenheit.

In analyzing results the logarithm of difference between the initial product and room air temperatures was computed. Since this quantity is a linear function of the cooling time (Fig. 1), it was only necessary to compute a single coefficient for temperature in setting up the equations. It was not known whether the cooling time would be a first or higher degree function of the weight. Theoretical considerations indicated that it would not be linear, but in view of the relatively high variability observed, the best fit could only be determined analytically.

The partial regression equations computed from the results are given (Table 1). Precooling in air was studied most extensively and two linear and second-degree equations were fitted to the data obtained by this method. Fewer birds were used in the experiments involving immersion in water, and some of the results had to be discarded owing to the suspected entrance of water into the body cavity. In consequence, only a limited amount of curve fitting seemed to be justified. The first experiment on precooling in air included a group of birds having a mean weight of 3.5 pounds rather uniformly distributed over the weight range from one to six pounds, as shown by the black portion of the inserted chart (Fig. 2). Birds of comparable size and weight distribution were used in both experiments involving immersion in water. The first experiment on air cooling is therefore directly comparable with those in which immersion was employed. Later, another experiment was conducted on precooling in air using heavier birds (Fig. 2, hatched portion) and the results of this study were combined with those of the first in obtaining Equations 2 and 4.

From the results of an analysis of variance to test the significance of the several equations (Table 2) it is evident that the regressions on temperature difference (initial product temperature) and body weight are highly significant when compared with the residual variance. The variance due to regression on weight squared (second-degree equations) is significantly greater than the residual variance

in the first experiment only. Since the variance accounted for by this term, although significant, was relatively small, and since the second-degree term contributed nothing in the combined experiments, it was concluded that a linear equation in terms of weight gives a satisfactory fit, considering the magnitude of the residual variance.

Equation 2, derived from the majority of the experimental data, was therefore used to construct Fig. 2, which shows the time required to cool birds of various weights over the temperature intervals likely to be met in practice. The temperature axis shows the difference in

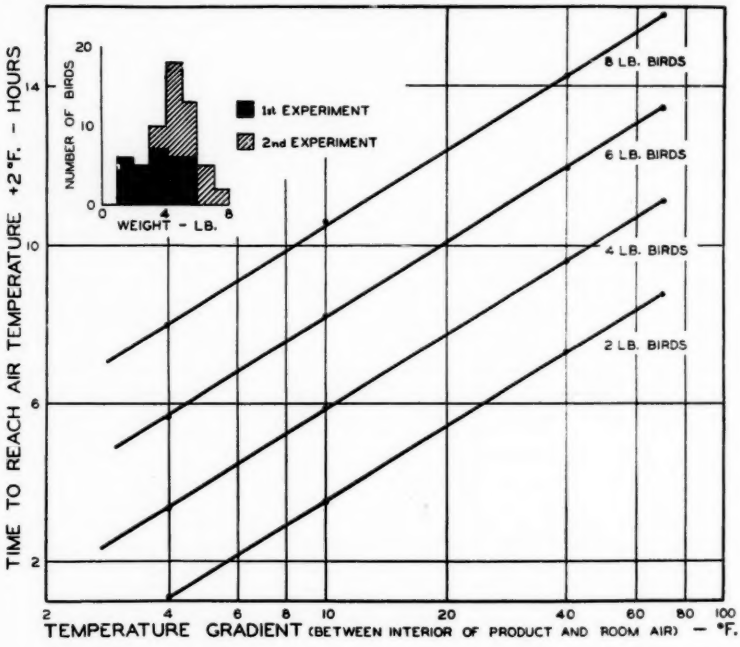

FIG. 2. Time required to cool birds of various temperatures and weights in air. (Insert shows weight distribution of birds used in establishing equations.)

temperature between the interior of the product and the air in the cooling room and has been plotted on a logarithmic scale to make the relation linear. The relatively large standard deviations, even after the effect of variable initial temperature and weight have been accounted for (Table 3), show the importance of other factors, such as shape of the bird, fat content, and deposition, in determining the time for cooling individual birds.

The mean weight of the birds used, the time required for cooling from their initial temperature to 1.1°C.(34°F.), and the standard

deviation of an individual bird from this mean time are reported (Table 3). Birds of 3.5 pounds in weight required a mean time of about five hours for cooling in air. A preliminary immersion in water at 7.2°C.(45°F.) before hanging in air at 0°C.(32°F.) had

TABLE 2

Analysis of Variance of Cooling Time in Air

Variance accounted for by	First experiment only		First and second experiments	
	Degrees freedom	Mean square	Degrees freedom	Mean square
Variable initial-product temperature..	1	36.758 [1]	1	37.328 [1]
Added effect of variable weight.....	1	8.998 [1]	1	157.348 [1]
Added effect of variable Wt.[2].....	1	4.715 [2]	1	0.000
Residual.....	26	0.821	55	3.233

[1] Significant 1% point. [2] Significant 5% point.

little effect on the time required to reach 34°F., although it did hasten cooling in the initial stages. Immersion in water at 32°F. for two hours before hanging reduces the time required to about 60 per cent of that required in air.

TABLE 3

Time Required for Precooling Poultry

Statistics	Birds precooled in air at 0°C.(32°F.)		Birds cooled in water at 32°F. for 2 hr. then in air at 32°F.	Birds cooled in water at 45°F. for 2 hr. then in air at 32°F.
	1st exp. only	1st and 2nd exp.		
	lb.	*lb.*	*lb.*	*lb.*
1. Mean weight of birds.....	3.5	4.4	3.6	3.6
	hr.	*hr.*	*hr.*	*hr.*
2. Mean time.....	5.0	6.9	2.86	4.9
3. Standard deviation (individual bird).....	1.6	2.5	0.74	.....
4. Standard deviation (individual bird) after allowing for effect of variable initial temperature.....	1.1	2.4	0.69	.....
5. Standard deviation (individual bird) after allowing for effect of variable initial temperature and weight by linear equation.....	1.0	1.8	0.57	.....

SHRINKAGE

Shrinkage was measured by weighing each bird separately on receipt at the cooling room and again 24 hours later at the time of packing. This made possible estimates of variability in shrinkage

between individual birds treated in the same way. Since the amount of shrinkage is determined by the factors that determine the rate of cooling, it was thought that the observed shrinkage might be related to the initial temperature and weight of the birds. When the results were analyzed, however, it was found that the variability in the shrinkage between individual birds was so great that it was impossible to establish these relations precisely.

The observed mean change in weight and the standard deviation for an individual bird are reported (Table 4). Two lots of birds were precooled in air under slightly different conditions and the

TABLE 4

Shrinkage of Poultry Precooled in Different Ways

Precooling conditions	Number of birds	Weight of birds	Mean change in weight	Standard deviation (individual bird)
		lb.	*pct.*	*pct.*
Convection cooled room, 32°F. Rel. hum. 70% at first, rising to over 80% for a few hours.	50	2-4	—0.24	0.12
	51	4-6	—0.22	0.09
Air stirred intermittently, 32°F. Rel. hum. initially 70%, rising to 75% for a few hours.	6	< 2	—1.10 [1]	0.40
	11	2-4	—0.78 [1]	0.15
	12	4-6	—0.41 [1]	0.15
Immersed in water at 32°F. or 45°F. for 2 hr. then hung in air as in experiment immediately above.	26	1.5-6	0.22	3.10

[1] Means significantly different.

results from each lot have been reported separately. Within each of the lots the birds have been classified according to their weight. The shrinkage observed in birds cooled by immersion in water at 32 and 45°F. for two hours before hanging in air is also reported for comparison. In this connection, it should be pointed out that the final weight was taken 24 hours after receipt at the room, when the birds were completely dried off and ready for packing.

The first experiment (Table 4) was conducted in a room at 32°F. without forced air circulation. The initial relative humidity was about 70 per cent and increased to over 80 per cent when the warm birds were placed in the room. At the time the final weights were taken the humidity was again about 70 per cent. No temperature observations were made on this group of birds. It is evident that the mean loss in weight was about one-fourth per cent and the loss in the two- to four-pound weight range was not significantly differ-

ent from that in the four- to six-pound weight range. Relative to this small loss the observed variations were quite large as shown by the standard deviations reported for individual birds. Some birds showed negligible weight losses while a few others showed losses in excess of one-half per cent.

The second experiment on cooling in air was made under the conditions described in discussing the precooling time. The slightly lower relative humidity with intermittent air circulation and the lower mean weight of the birds probably all contributed to a greater loss in weight, the mean value being about three-fourths per cent Under these conditions it was possible to demonstrate that the percentage of shrinkage decreased as the weight of the birds increased, the differences between the shrinkage of the different weight classes being statistically significant (Table 4).

Since the evaporation of moisture from the product also aids in cooling, it is of interest to determine what proportion of the heat was removed in this way under the two sets of conditions used. At one-fourth per cent shrinkage 100 pounds of product would lose one-fourth pound of water, equivalent to the removal of about 265 B.T.U. Since the specific heat of the product is about .8, the removal of this amount of heat would cool this mass of product somewhat over three degrees Fahrenheit. Product suffering one-half per cent shrinkage would be cooled about seven degrees F. by evaporation. Since the majority of the birds were cooled over 50 degrees F., it is obvious that evaporation does not contribute over about 15 per cent of the cooling at the most.

In the experiments involving immersion in water for two hours prior to hanging in air, the fowls showed a mean gain in weight of 2.2 per cent after hanging 22 hours in air and the surfaces were completely dry. This value was obtained after eliminating all the birds in which the leakage of water into the body cavity around the thermocouple leads was suspected. Nevertheless, the relatively large gain in weight, combined with the enormous variability between different birds, suggests that water may have entered the body cavity through other channels.

In conclusion it appears that a 24-hour precooling period in air, under conditions approximating those found in commerce, will cause an average shrinkage of about one-fourth per cent. Lower relative humidities than those used in these tests or forced air circulation will increase the shrinkage. As might be expected, shrinkage depends on the size of the product. Light birds suffer a greater shrinkage than heavy birds; those below two pounds may lose one per cent during cooling. Immersion in water for two hours prior to hanging

in air, results in a gain in weight of about two per cent, probably owing to the retention of water in the body cavity. The loss or gain of an individual bird deviates greatly from the mean values reported; some show negligible change while others may gain or lose over twice the average amount.

BACTERIAL CONTENT OF WATER USED FOR PRECOOLING

Where temporary immersion in water is used to hasten the cooling rate, it is possible that the water may also be a source of contamination and, by increasing the bacterial load on the surface of the product, may reduce its safe storage life in the chilled state, according to Lochhead and Landerkin (1935). Storage experiments at temperatures above the freezing point were not conducted nor was the bacterial content of product investigated, but some preliminary

TABLE 5

Bacterial Content of Water Used for Precooling

Date of sampling	Lot No.	Water at 0°C.(32°F.)		Water at 7.2°C.(45°F.)	
		Number of bacteria per ml. of water		Number of bacteria per ml. of water	
		Before immersion Log 10	After immersion Log 10	Before immersion Log 10	After immersion Log 10
1/10/36	1	2.2	2.3	3.6	3.4
2/10/36	2	2.1	2.4	4.1	3.8
5/10/36	3	2.4	2.6	4.4	4.5
6/10/36	4	2.4	2.7	7.2	7.3
9/10/36	5	2.6	3.0	5.6	6.0

measurements were made on the bacterial content of the water in the tanks used for precooling. Samples were taken immediately before and after immersing each lot of birds, and as the same water was used for all lots, the results also give some indication of the changes likely to occur on standing. These changes must be interpreted with caution, however, since the time interval between successive immersions and counts varied somewhat, and the temperature was not closely controlled during these intervals. The bacterial numbers were estimated by plating appropriate dilutions on Bacto-nutrient agar and incubating them for 48 hours at 20°C.(68°F.) before counting.

The logarithms of the bacterial numbers observed in the tanks at 0 and 7.2°C.(32 and 45°F.) are given (Table 5). The same water was used in both tanks and the higher initial count in the tank at 45°F. must be attributed to the growth which occurred during the period of temperature adjustment prior to making the first count.

It can be seen that the immersion of each lot of birds generally tended to increase the bacterial count slightly at both temperatures, but this increase cannot be regarded as serious in relation to the number originally present. It is also evident that the bacterial content of the water in the tank at 32°F. increased somewhat over the entire experiment, while the water at 45°F. increased markedly. In commercial practice the water would have to be changed frequently to maintain the required temperature, and this gradual increase would be unlikely to occur. At the same time these results indicate that a water temperature near the freezing point is desirable in order to keep the bacterial count as low as possible.

EFFECT OF PRECOOLING ON BLOOM AND APPEARANCE

Some operators feel that the temporary immersion of poultry in water may be detrimental to the bloom or appearance. This point was investigated by packing five boxes, each of which contained six birds precooled by immersion in water for two hours followed by hanging in air, and six birds precooled in air only. Since bacterial activity limits the storage life to a few weeks at temperatures above the freezing point, they were stored in the frozen state at —13.6°C. (7.5°F.). The birds were examined at the time of packing and periodically thereafter for 139 days, when the surfaces were so badly desiccated (freezer-burned), that further observations were useless. The usual commercial boxes and wax-paper liners were used throughout.

Two inspectors examined and scored the individual birds for bloom at each examination. Five grades of bloom were defined in advance and each allotted a whole number which increased with the quality. As the breasts and legs were scored separately, a score of ten was possible for each bird. These individual scores were later summed for the six birds precooled by each method in each box. These summed scores were used in making all subsequent calculations and comparisons.

Before presenting the results it is necessary to discuss the arbitrary nature of such subjective observations. It was impossible to compare individually each of the 60 birds used in the experiment. In the first place the birds were frozen and could not be moved and classified separately. Secondly, the five boxes of birds varied in net weight from about 20 to 63 pounds and, as the quality and finish is somewhat dependent on the weight, it was difficult to assess the differences in bloom among birds that varied so greatly in over-all quality. In consequence, each box was scored as an individual lot, and the five boxes may be regarded as replications. In scoring, the

practice was to select at each examination one or two birds in each box that seemed to be representative of one of the defined scores. This bird or birds, was then taken as a standard in scoring the remaining birds. By this method it was possible to place certain birds in an intermediate position between two defined grades, and these birds were given the score for the lower grade plus a half.

It is not necessary to consider the individual scores by birds or boxes in detail. As the birds were chosen at random for this investigation, the over-all quality of the product varied considerably between birds, between lots precooled in water and air and packed in the same box, and between boxes. This was reflected in the scores at all examinations. The general trend was for bloom to decrease

TABLE 6

Comparison of Precooling in Air and Water on Subsequent Bloom of Birds in Frozen Storage at —13.6°C.(7.5°F.)

Box No. and weight (net)			Ratio bloom scores of birds precooled in water and in air				
			Initial after freezing	After 31 days' storage	After 65 days' storage	After 92 days' storage	After 138 days' storage
	lb.	*oz.*					
1	19	13	110	115	116	104	129
2	35	8	106	103	102	101	112
3	44	6	113	118	91	100	105
4	51	10	91	96	91	92	72
5	62	14	100	92	99	100	109

Differences	Degrees freedom	Mean square
Within examinations	20	150.5
Between examinations	4	53.8

with increasing time in storage. There was also a tendency for the boxes having the poorest bloom initially to decrease most. The scores allotted at one examination were consistently higher than those allotted on the previous and subsequent examinations, and the differences between the several boxes were also less marked on that occasion. This result can be attributed to the subjective nature of the scoring, and casts doubt on the significance of the quantitative estimates of the decrease of bloom with time.

Since birds precooled by the two methods under test were packed in the same box, and all compared with a bird in that box as a standard, the effect of the two precooling treatments can be considered independent of variations in the inspectors' judgment affecting comparisons between boxes or between inspections. The scores of the birds precooled in water were expressed as a percentage of those

precooled in air for all boxes and observations (Table 6). The departure of this ratio from 100 per cent in the initial examination merely indicates the variable quality of the birds precooled by the two methods.

These results were treated statistically by computing the variance within and between examinations. The variance within examinations may be taken as an estimate of the "experimental" error, while that between examinations, i.e., with time, would show any differential effect of storage on the birds precooled by the two methods. From the results it is obvious that the variance within examinations, which results from the variable initial quality of the product and the inspectors' judgment, far exceeds the differences arising during storage (Table 6, lower part). It is therefore concluded that if any differential effect does occur, it is negligible compared with the variations ordinarily observed between birds similarly treated. This conclusion is in agreement with the opinion of practical inspectors who examined the product at the final inspection and stated that the evident differences were due to the quality of the birds themselves rather than to the different treatments.

SUMMARY

Equations are given relating the initial-product temperature, air temperature, and weight of the bird to the average time required for cooling to any required temperature above the freezing point. The importance of certain unknown properties of the product in determining the time required for precooling is demonstrated by the relatively large residual variance, after accounting for the effect of temperature gradient and weight by the above equations.

Immersing the birds in water at 7.2°C.(45°F.) for two hours prior to hanging in air at 0°C.(32°F.) has little effect on the over-all time required for cooling. A similar treatment using water at 32°F. reduces the time to about 60 per cent of that required in air alone. The loss in weight during cooling in air varies, on the average, from one-fourth to one-half per cent, depending on the atmospheric conditions. Individual birds deviate considerably from these mean values. Immersion in water for two hours results in a mean over-all gain in weight of extremely variable magnitude. The bacterial content of the water used for immersing the birds was much higher in the tank at 45°F. than in the tank at 32°F. No evidence was obtained to indicate that temporary immersion in water during precooling had either a detrimental or beneficial effect on the retention of bloom during subsequent storage in the frozen state.

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SURVIVAL OF THERMOPHILIC FOOD-SPOILAGE ORGANISMS IN STORED WHITE BEET SUGAR[1]

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The establishment of bacterial standards for canning-grade sugar by the National Canners Association has encouraged the production of bacteria-free sugar, and concerted efforts are being made by manufacturers to prevent contamination during its manufacture, storage, and handling.

The literature concerning the presence and significance of thermophilic canned-food spoilage organisms in white sugar has become rather voluminous since their presence was first reported by Cameron and Williams (1928, 1929). Numerous workers, including James (1928), Cameron (1930), Cameron and Bigelow (1931), Cameron and Yesair (1931), and Homans (1933), have discussed thermophilic bacteria from the standpoint of their origin in sugar. Owen and Mobley (1932) have discussed the elimination of bacteria from sugar refineries, while more recently Calton (1936) has discussed the elimination of thermophilic bacteria from beet sugar during its manufacture.

The complete elimination of bacterial spores from white sugar during processing is beset with many difficulties, but by continual surveillance and careful control of operations most processors are consistently able to produce sugar that is acceptable to canners. Frequently, however, lots of sugar are produced which, according to the National Canners Association standards, are contaminated with sufficient spores to make its use for canning undesirable. Sometimes the number of spores only slightly exceeds the tolerance, thus making use of the sugar questionable.

In guaranteeing the bacteriological quality of sugar the manufacturer must consider the possibility of changes in the numbers of food-spoilage organisms owing to multiplication or death of the original contamination. The factor of death may be particularly significant where bacterial counts bordering the maximum number of spores permitted are involved at the time of manufacture. The fate of microörganisms in stored sugar has not been extensively investigated; however, Browne (1918) observed that during a study of

[1] Food Research Division Contribution No. 396.

microbial deterioration of raw cane sugar the microörganisms do not survive long periods of storage. Browne states that "on very long storage the number of microörganisms undergoes a decrease; samples of raw sugar after two years are frequently found to be almost sterile."

CONTAMINATION IN SUGAR

Three groups of thermophilic bacteria are recognized in sugar as being significant in the spoilage of canned foods: (1) the 'flat-sour" spoilage organism, of which *Bacillus stearothermophilus*, Donk (1920), is the type species; (2) thermophilic anaerobes, with type species, *Clostridium thermosaccharolyticum*, McClung (1935); and (3) sulfide-spoilage anaerobes of which the type species is *Clostridium nigrifaciens*, Werkman (1929). Standards have been established for the groups by the National Canners Association, given by Cameron (1930) and Bigelow (1931), for judging the suitability of sugar for canning purposes. The standards are based on the examination of five samples from each lot of sugar and are as follows:

Total Thermophilic-Spore Count: For the five samples examined there shall be a maximum of not more than 150 spores in any one sample and an average of 125 spores per 10 grams of sugar.

Flat-Sour Spores: For the five samples examined there shall be a maximum of not more than 75 spores in any one sample and an average of not more than 50 spores per 10 grams.

Thermophilic Anaerobic Spores: These shall be present in not more than three (60 per cent) of the five samples and in any one sample to the extent of not more than four (65+ per cent) tubes.[2]

Sulfide-Spoilage Spores: These shall be present in not more than two (40 per cent) of the five samples and in any one sample to the extent of not more than five spores per 10 grams.

Each of the past several years the National Canners Association laboratories have reported a summary of the results of the bacteriological examination of a large number of beet- and cane-sugar samples. For the years 1936 and 1937 they report that all beet-sugar samples tested were within the standards established for canning-grade sugar.

During the beet-sugar campaign years, 1935 to 1937 inclusive, 203 campaign-composite samples were obtained by this Bureau from all operating domestic factories and were tested according to methods published by Cameron (1936) to determine suitability as canning-grade sugar. The results, based on the number of samples within and below the standards, are given (Table 1).

[2] Method of testing.

A yearly improvement in the quality of domestic beet sugar is shown for the past three years. In 1937 only six (8.6 per cent) of 70 operating factories did not manufacture canning-grade sugar. This is in contrast to 10 (14.3 per cent) of 70 factories in 1936 and 17 (26.2 per cent) of 65 factories in 1935 that failed to manufacture canning-grade sugar. The fact that these results indicate more sugar

TABLE 1

Thermophilic Contamination in Campaign-Composite Beet-Sugar Samples From Individual Factories

Year	Total samples	Within standard		Below standard		Samples below standard because of			
						Total count	F.S.[1]	T.A.[2]	S.S.[3]
			pct.		*pct.*				
1935	65	48	73.8	17	26.2	11[4]	17	0	0
1936	70	60	85.7	10	14.3	5[4]	10	0	0
1937	70	64	91.4	6	8.6	2[4]	5	1	1[5]

[1] F.S. = Flat-sour bacteria. [2] T.A. = Thermophilic anaerobes. [3] S.S. = Sulfide-spoilage anaerobes. [4] Also contained excessive F.S. spores. [5] Also contained excessive T.A. spores.

of non-canning grade than the National Canners Association reports show may be due to their failure to receive samples from all operating factories.

Although the above results show that the bacterial content of the campaign-composite samples from a few factories exceeded the stand-

TABLE 2

Contamination in Consecutive Individual and Composite Strike Samples

Factory	Consecutive individual samples			Consecutive composites of 25 samples each		
	A	B	C	A	B	C
Flat-sour spores per 10 grams.	10	15	0	0	30	5
	10	25	20	0	20	35
	0	40	0	5	5	10
	0	85	10	0	5	10
	15	5	10	0	10	45
	5	....	165	0	....	70

ards, this does not mean that all sugar manufactured by those factories contained excessive numbers of bacteria. Variations have been found to occur in the bacterial content of consecutive individual strikes and in consecutive composite samples of 25 strikes each. As an illustration of this the results of the examination of individual and composite samples for flat-sour-type spores, which predominate in contaminated samples, are given (Table 2).

Two of the 17 consecutive individual strike samples contained in excess of 75 spores per 10 grams of sugar as permitted for individual samples. The sample containing 165 spores would be rejected for canning purposes. One of these samples, that containing 85 spores, may be classified as a border-line sample and strict interpretation of the standards would thus make use of such sugar for canning purposes questionable. One of the composite samples contained in excess of 50 spores per 10 grams permitted for composite samples. Its use for canning purposes would probably not be considered since, in order to obtain an average of 70 spores, several of the individual samples making up the composite would contain excessive numbers of spores.

Flat-sour-type spores were found to predominate in contaminated samples. They were present in 24 (70.6 per cent) of the above 34

TABLE 3

Thermophilic Bacteria Content of Sugar Stored Eight Months in Paper, Burlap, and Toweling Bags

Spore count per 10 grams sugar	Paper		Burlap		Toweling	
	1-14-37	8-30-37	1-14-37	8-30-37	1-14-37	8-30-37
Total aerobes (av.)	7.5	5	10	10	7.5	10
Flat sour (av.)	7.5	5	10	10	7.5	10
Thermophilic anaerobes	1+, 5—	6—	6—	6—	6—	6—
Sulfide spoilage	0	0	0	0	3.0	0

samples. Other type aerobic spores, which contribute to the total aerobic spore count, are often present with the flat-sour-type spores but are seldom the sole aerobic contaminant. Anaerobic spores were present in 19 per cent of 331 composite samples examined and only two per cent contained excessive numbers of spores. In view of these results it is believed that the determination of the presence and numbers of aerobic spores, especially the flat-sour type, may be used as a reliable index of the bacteriological quality of white sugar. This may be especially true while testing sugar during the manufacturing period when large numbers of samples must be handled.

EXAMINATION OF STORED SUGAR

Approximately 100 samples of sugar, taken from different factories and representing different variations in methods of purification, were packaged in paper, burlap, toweling, and glass containers and stored in sugar warehouses and in the laboratory for various periods of time. The samples that were stored in warehouses were packaged in 100-pound paper, burlap, and toweling bags. Smaller samples (one to 10 pounds) were packaged for laboratory storage

in sealed sterile glass and paper containers in order to conserve space and to exclude the possibility of outside contamination.

The samples were examined bacteriologically soon after being manufactured and after approximately eight and 20 months' storage. The storage period of eight months represents the time that beet sugar is usually stored for canning purposes. In years of large carry-over of sugar stocks the storage period might be 20 months. Typical results of the examination in duplicate of low-spore-count sugar samples from a single strike packaged in paper, burlap, and toweling and stored for eight months in a sugar warehouse are given (Table 3).

The initial bacterial content of this lot of sugar was very low, there being present a maximum of only 10 aerobic spores per 10 grams of sugar, of which all were of the flat-sour type. Sugar having a higher initial spore content would have been more desirable for warehouse storage, but such was not available at the time the experiment was started. There was no marked increase or reduction in the aerobic bacteria content of the sugar; however, it was not possible to demonstrate the presence of the original anaerobic contaminants in the sugar after eight months of storage. The slight variations shown in the spore content of the sugar after storage are well within the limits of variation that might be expected for the test methods and can probably be dismissed as being significant. The fact that the spore content did not show a marked increase during the storage period is an indication that when few thermophilic bacteria are present in sugar they do not multiply during storage.

A large number of samples were selected for storage in paper and glass containers, which contained various numbers of aerobic-type spores. The samples were stored in the laboratory for seven to eight and 20 months, after which time examination was again made to determine the spore content. The original contamination of spores and the numbers present after storage, together with the percentage of increase or decrease are shown (Tables 4 and 5).

There was a decrease in the number of spores in 35 (87.5 per cent) of 40 samples included in the two tables. The reduction of spores in sugar packaged in paper bags was from 11.1 to 85.7 per cent of the total aerobic spores and from 11.1 to 92.8 per cent of the flat-sour-type spores. In the samples packaged in the glass containers, the reduction of total spores was from 5.4 per cent in a sample stored for 20 months to 100 per cent in a sample stored only eight months. The reduction of flat-sour-type spores was from 6.8 per cent to 100 per cent. One sample which did not originally contain spores was found to be free of them after eight months' storage.

Four samples showed increased numbers of spores after storage. The greatest increases were in originally low-spore-count samples and were from 27.2 per cent to 400 per cent for total spores and from 16.6 per cent to 300 per cent for flat-sour-type spores. These increased spore counts are believed to be due to errors in testing rather than to actual multiplication of the original contamination. The absence of strong acid-producing spores was noted in the stored

TABLE 4

Survival of Aerobic Thermophilic Spores in Sugar Stored in Paper Bags

Sample	Storage period	Spore count per 10 grams				Increase or decrease	
		Original		Final			
		Total	F.S.[1]	Total	F.S.	Total	F.S.
	mo.					*pct.*	*pct.*
1	8	0	0	0	0	0	0
2	7	5	5	25	20	400.0	300.0
3	8	35	25	5	5	—85.7	—80.0
4	7	60	45	40	40	—33.3	—11.1
5	8	70	60	25	20	—64.2	—66.6
6	8	70	70	25	5	—64.2	—92.8
7	8	85	30	60	35	—29.4	16.6
8	8	135	80	120	60	—11.1	—25.0
9	8	150	145	100	100	—33.3	—31.0
10	8	565	400	325	140	—42.4	—65.0
11	20	20	15	5	5	—75.0	—66.6
12	20	40	10	15	10	—62.5	0.0
13	20	45	40	35	35	—22.2	—12.5
14	20	75	65	40	30	—46.6	—53.8
15	20	90	75	30	30	—66.6	—60.0
16	20	110	75	140	120	27.2	60.0
17	20	120	90	70	65	—41.6	—27.7
18	20	220	155	160	125	—27.2	—19.3
19	20	395	325	230	210	—41.7	—35.0
20	20	950	875	385	355	—59.4	—59.4

[1] F.S. means flat-sour type.

samples, indicating a possible loss of this property in spores during storage. This point needs further and more careful study.

Although duplicate samples were not used for storage in this experiment, the results, based on comparative original counts, indicate that the type of container did not influence the survival of spores. The reduction of spores in samples stored for 20 months does not appear to be correspondingly greater than in samples stored for eight months. This would indicate that the greatest initial reduction of spores might be expected to occur during the first months of storage.

The reason for the failure of spores to survive in stored sugar is not known. It is suggested, however, that since sugar is subjected to a drying process in the final stages of manufacture, dehydration of weak spores occurs to such an extent that they gradually die, the stronger spores possibly surviving longer periods of storage. Also, sugar impurities having hygroscopic properties might cause prolonged desiccation of spores, causing weakening or eventual death.

TABLE 5

Survival of Aerobic Thermophilic Spores in Sugar Stored in Glass Containers

Sample	Storage period	Spore count per 10 grams				Increase or decrease	
		Original		Final			
		Total	F.S.[1]	Total	F.S.	Total	F.S.
	mo.					*pct.*	*pct.*
1	8	20	20	15	15	—25.0	—25.0
2	8	35	15	0	0	—100.0	—100.0
3	8	50	25	30	15	—40.0	—40.0
4	8	55	25	45	15	—18.1	—40.0
5	8	70	40	70	5	0.0	—87.5
6	8	80	35	45	5	—43.7	—85.7
7	8	95	75	40	25	—57.8	—66.6
8	8	145	65	110	50	—24.1	—23.0
9	8	150	115	100	80	—33.3	—30.4
10	8	165	100	40	5	—75.7	—95.0
11	20	5	5	25	15	400.0	200.0
12	20	45	30	0	0	—100.0	—100.0
13	20	55	25	5	5	—90.9	—80.0
14	20	60	55	35	30	—41.6	—45.4
15	20	90	75	40	40	—55.5	—46.6
16	20	105	90	0	0	—100.0	—100.0
17	20	120	105	105	85	—12.5	—19.0
18	20	150	115	105	20	—30.0	—82.6
19	20	155	45	5	5	—96.7	—88.8
20	20	370	295	350	275	—5.4	—6.8

[1] F.S. means flat-sour type.

The possibility of known impurities in sugar influencing the reduction of spore counts was investigated. Chemical analysis of an equal number of samples that were stored in paper and glass containers for 20 months were made by the Carbohydrate Research Division [3] for ash, sulphate, and sulphite content, and pH values of the sugar; the results of the examination for spores before and after storage and the analysis are given (Table 6).

A comparison of the reductions of the number of spores with the ash, sulphate, and sulphite content does not reveal any general

[3] Analysis made by S. A. Byall, Carbohydrate Research Division, of this Bureau.

relationship between the amounts of these impurities and the decreased spore counts. Neither is there a relationship between the pH values of the sugar and the reductions.

SUMMARY AND CONCLUSIONS

Thermophilic food-spoilage organisms in beet sugar stored in paper, burlap, toweling, and glass containers were generally found to undergo a decrease in numbers during storage for eight and 20 months. Greatly increased reductions of spores does not occur in sugar with comparable initial counts after the first eight months

TABLE 6

Reduction of Spores in Sugar With Known Impurities

Sample	Spores per 10 grams				Decrease		Impurities			pH
	Original		Final							
	Total	F.S.[1]	Total	F.S.	Total	F.S.	Ash	SO_3	SO_2	
					pct.	*pct.*	*pct.*	*p.p.m.*	*p.p.m.*	
1	45	40	35	35	22.2	12.5	.0153	2.0	12.5	6.9
2	75	65	40	30	46.6	53.8	.0178	1.0	14.7	6.8
3	90	75	30	30	66.6	60.0	.0114	1.0	12.2	6.8
4	120	90	70	65	41.6	27.7	.0190	0.0	21.8	6.7
5	395	325	230	210	41.7	35.0	.0153	1.0	25.0	6.6
6	950	875	385	355	59.4	59.4	.0203	11.0	10.2	6.9
7	45	30	0	0	100.0	100.0	.0058	0.0	1.3	6.7
8	60	55	20	20	66.6	63.6	.0118	5.0	2.6	6.7
9	90	75	40	40	55.5	46.6	.0114	1.0	12.2	6.8
10	105	90	0	0	100.0	100.0	.0098	0.0	14.1	6.8
11	120	105	105	85	12.5	19.0	.0148	1.0	5.1	6.8
12	155	45	5	5	96.7	88.8	.0190	4.0	6.7	7.5

[1] F.S. means flat-sour type.

of storage, indicating that the most rapid reduction is soon after the sugar is manufactured. The type of container does not seem to influence the survival of organisms in sugar during storage.

A comparison of the decreased spore counts with known impurities in sugar was made to determine if a relationship existed between spore reductions and impurities. There was no relationship demonstrated. It is suggested that decreased spore counts result from dehydration caused by drying of sugar during manufacture or from the presence in sugar of hygroscopic impurities. Storage of sugar for several months after its production often results in the fatality of sufficient spores to improve its quality to that of canning-grade sugar. Storage seems to offer possibilities in commercial practice as a means of improving the bacteriological quality of sugar.

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THE ROLE OF SPICES IN PICKLED-FOOD SPOILAGE[1]

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Spices are an integral part of the flavoring in many food products. Since earliest times they have been associated with foods and have been considered in the role of preservatives along with salt, sugar, and acids, despite the fact that Leeuwenhoek found millions of microörganisms in an infusion of whole pepper as early as the seventeenth century. The significance of Leeuwenhoek's findings has been completely overlooked and during the intervening centuries practically all the work that has been done with spices has been done on the action of spices in preventing rather than causing spoilage in foods to which they were added. Much work has been done to show the antimicrobial action of the various spices separately and in combination with foods.

Several years ago in a series of experiments on pickles which were set up to determine the cause of spoilage, it became evident that the mixed spices used were responsible for the spoilage which occurred since all the other factors were controlled or eliminated. During the past year the experiments were repeated, and they confirmed the earlier results.

In the experiments reported here bacteriological tests were made on whole mixed spices, whole spices, ground spices, the oil of spices, and emulsions of the oils. Experiments were made also to study the influence of contaminated spices on food spoilage in pickled products, such as pickles, pears, and peaches, as well as the antibacterial properties of various spices.

A total of 32 whole spices from 12 different sources, 22 ground spices from 12 different sources, seven soluble sugar-base spices from one source, 12 oils of spices from one source, and 10 oils of spices in a 50-per cent emulsion from one source were tested during the course of this work. It is believed that the results of the bacterial analyses presented here represent a fairly good cross section of the general condition that one might expect to find in spices collected on the open market.

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[1] Journal article No. 335 n.s.

BACTERIAL CONTENT OF WHOLE MIXED SPICES

The whole mixed spices were tested bacteriologically according to the following procedure: One gram of the whole mixed spice was weighed out on sterile filter paper and added to 99 ml. of sterile water. The mixture was shaken 25 times and suitable dilutions plated from this. In order to determine whether or not most of the bacteria were removed from the spices by this method, a variation of this procedure was tried by allowing the spices to soak in the sterile water for 30 minutes and then shaking before plating them. No significant differences were noted in the bacterial count between the two meth-

TABLE 1

Bacterial Content per Gram of Whole Mixed Spice and the Individual Constituent Spices

Spice	Total bacterial count	Acid colonies	Peptonizers	Alkali and inert colonies
Whole mixed spice	67,000,000	9,000,000	7,000,000	51,000,000
Allspice [1]	130,000	30,000	40,000	60,000
Peppercorn	17,000,000	500,000	11,000,000	5,500,000
Red pepper	40,000	3,000	7,000	30,000
Bay leaf	80,000	7,000	20,000	53,000
Cardamon	96,000	4,000	4,000	88,000
Nutmeg	1,200,000	10,000 [2]	20,000 [2]	1,170,000
Cinnamon	28,000,000	300,000 [2]	100,000 [2]	27,600,000
Orris root	12,600,00	1,300,000	2,100,000	9,200,000
Mustard seed	480,000	30,000	30,000	420,000
Small brown seed	133,000	10,000	13,000	110,000
Small black seed	1,190,000	30,000	30,000	1,130,000

[1] This and the following spices were separated from the whole mixed sample above.
[2] Very small colonies.

ods. This would indicate that the bacteria were mostly on the surface of the spices.

The medium used for plating was sodium caseinate agar to which had been added one per cent of glucose and bromecresol purple as the indicator. This medium permitted a differentiation of acid-producing bacteria, peptonizers, and alkali and inert bacteria as well as the obtaining of the total count. In the first experiment the bacterial content of a whole mixed spice was determined and then the individual spices were separated from the mixed spice and a bacterial analysis made of them. This was done in order to determine which individual spice was contributing the largest number of bacteria.

From the results it is evident that whole mixed spices may contain large numbers of bacteria (Table 1). It is significant that there was over 10 per cent peptonizers present. When individual spices

were separated, cinnamon had the largest number of bacteria followed by peppercorn, orris root, and nutmeg. It was this mixed spice that was used in subsequent experiments (Tables 8 to 13).

A bacterial analysis was made of samples of whole spices to determine the extent of the contamination of this type of spice as found on the open market. Some samples were labeled "sterile" on the container. All so labeled were found to be sterile. There was no statement regarding the sterility of the rest of the whole spices tested. They were found to contain from a few hundred to several millions of organisms. None of the samples of whole spices tested

TABLE 2

Bacterial Content per Gram of Whole Spices

Spice	Label designation	Total bacterial count	Acid colonies	Peptonizers	Alkali and inert colonies
Cloves	Sterile	0	0	0	0
Cloves	Sterile	0	0	0	0
Celery seed	Not indicated	320,000	100,000	10,000	210,000
Celery seed	Not indicated	380,000	100,000	10,000	250,000
Celery seed	Sterile	0	0	0	0
Mustard seed	Sterile	0	0	0	0
Allspice	Sterile	0	0	0	0
Peppercorn	Not indicated	1,500,000	170,000	470,000	860,000
Peppercorn	Not indicated	2,200,000	200,000	500,000	1,500,000
Bay leaf	Not indicated	9,300	1,850	250	7,200
Bay leaf	Not indicated	10,000	2,600	500	6,900
Poppy seed	Not indicated	1,600	100	250	1,250
Caraway seed	Not indicated	2,000	300	400	1,300
Caraway seed	Not indicated	23,000	1,600	1,600	19,800
Caraway seed	Not indicated	25,000	2,400	1,600	21,000

had as many organisms present as the whole mixed spice (Table 1). The results of the analyses of whole spices are given (Table 2).

There were many more bacteria present in the ground spices than in the whole spices (Table 3). Only one of the total number tested was sterile. This would indicate that spices may be contaminated during the process of grinding. The ground chile mix contained the greatest number and the white pepper the smallest.

The following spices dissolved in a sugar base were found to be sterile: red pepper, allspice, cinnamon, cloves, dill, ginger, and mace. Likewise the oils of the following spices were found to be sterile: cloves, mustard (natural), mustard (artificial), dill weed, caraway, coriander, cassia (U.S.P.), sassafras, lemon, anise, orange, and cassia (Technical). Fifty-per cent emulsions of the following

spices were also found to be sterile: ginger, nutmeg, mustard, allspice, celery seed, cassia, cloves, dill, coriander, and caraway.

ANTIBACTERIAL ACTION OF SPICE SOLUTIONS

To determine the antibacterial action of spice infusions, the following whole spices—allspice, red pepper, orris root, mustard seed, celery seed, cinnamon bark, peppercorn, bay leaf, cinnamon stick, and cloves—were weighed in 10-gram quantities and made up to 100 ml. with distilled water. These were then autoclaved in closed

TABLE 3

Bacterial Content per Gram of Ground Spices

Spice	Total bacterial count	Acid colonies	Peptonizers	Alkali and inert colonies
Cloves	0	0	0	0
Celery	4,000,000	1,550,000	2,100,000	950,000
Mustard	161,000	10,000	1,000	150,000
Mustard	50,000	7,000	3,000	40,000
Mustard (rancid)	700,000	2,000	13,000	685,000
Mustard (old)	1,600,000	300,000	100,000	800,000
Mustard (fresh)	810,000	100	200	807,000
Mustard	560,000	10,000	10,000	540,000
Allspice	500,000	100,000	260,000	140,000
Allspice	1,360,000	300,000	800,000	260,000
Allspice	730,000	180,000	350,000	200,000
Ginger	580,000	150,000	35,000	395,000
Jamaica ginger	1,700,000	160,000	460,000	1,080,000
Paprika	600,000	200,000	60,000	340,000
Paprika	65,000	16,000	39,000	11,000
Chile mix	10,600,000	1,600,000	3,000,000	6,000,000
Mace	24,000	9,000	700	14,300
Cinnamon	30,000	11,200	150	18,600
Marjoram	150,000	17,000	30,000	103,000
White pepper	1,600	300	700	6,000
Turmeric	850,000	60,000	340,000	450,000

bottles in order to sterilize them and to extract the oil. From this 10-per cent infusion, dilutions of 1:50 and 1:100 were made in broth and inoculated with 24-hour broth cultures of the following organisms: stock cultures of *Bacillus subtilis*, *Escherichia coli*, and *Eberthella typhosa* (Hopkins strain), *Staphylococcus aureus* (Strain 141 isolated from chocolate éclair causing food poisoning 6-6-33), and four aerobic sporogenic bacteria isolated previously from spices and to which had been assigned the numbers 2, 3, 7, and 8. All readings were made after incubation at room temperature for seven days except *Staphylococcus aureus*, *Eberthella typhosa*, and *Escherichia*

coli, which were incubated at 37°C.(98.6°F.) for two days and then for five days at room temperature.

There was growth of all organisms in the 1:50 dilution of all spice infusions except that of cinnamon in which organism No. 8 failed to grow and of cloves in which only the two gram-negative, asporulat-

TABLE 4

Growth of Bacteria in Broth Containing Different Dilutions of an Infusion of Cinnamon

Organism [1]	Dilutions in broth					
	1:100	1:200	1:400	1:600	1:800	1:1,000
No. 2	2+	2+	2+	2+	2+	2+
No. 3	+	+	+	+	+	+
No. 7	2+	2+	2+	2+	2+	2+
No. 8	—	+	+	+	+	+
Bacillus subtilis	+	2+	2+	2+	2+	2+
Staphylococcus aureus	+	+	2+	2+	2+	2+
Eberthella typhosa	+	+	2+	2+	2+	2+
Escherichia coli	+	+	+	+	+	+

[1] — = no growth; + = moderate growth; 2+ = abundant growth.

ing bacteria, *Eberthella typhosa* and *Escherichia coli*, grew. All the gram-positive bacteria failed to grow. In the 1:100 dilution of cinnamon, organism No. 8 was the only one of the group which failed to grow; whereas in cloves, organisms No. 7 and 8, *Bacillus subtilis* and *Staphylococcus aureus*, failed to grow. This would indicate that

TABLE 5

Growth of Bacteria in Broth Containing Different Dilutions of an Infusion of Cloves

Organism [1]	Dilutions in broth					
	1:100	1:200	1:400	1:600	1:800	1:1,000
No. 2	2+	2+	2+	2+	2+	2+
No. 3	+	+	+	+	+	+
No. 7	—	—	+	+	+	+
No. 8	—	—	—	—	—	—
Bacillus subtilis	—	2+	2+	2+	2+	2+
Staphylococcus aureus	—	—	—	—	—	+
Eberthella typhosa	+	2+	2+	2+	2+	2+
Escherichia coli	2+	2+	2+	2+	2+	2+

[1] — = no growth; + = moderate growth; 2+ = abundant growth.

cloves had a greater antibacterial action than cinnamon. From these results it was evident that the water infusions of cloves and cinnamon were the only ones having any antibacterial properties. Experiments were then continued with these two, using more dilute solutions (Tables 4 and 5).

TABLE 6

Antibacterial Action of Different Percentages of Ground Spices in Nutrient Agar

Spice	Organism																	
	No. 2			No. 3			No. 7			No. 8			*Bacillus subtilis*			*Staphylococcus aureus*			*Eberthella typhosa*			*Escherichia coli*		
	A	B	C[1]	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
.1 per cent																								
Allspice	.....			2	—	0	2	—	0	.....			.....			1	—	½	.....			.....		
Peppercorn	.....			.....			.....			.....			.....			2	—	0	.....			.....		
Pepper	.....			.....			.....			.....			2	1	0	2	1	0	.....			.....		
Cloves	2	—	¾	2	—	¾	2	—	¾	2	—	¾	2	—	¾	2	—	¾	2	—	¾	2	—	¾
Nutmeg	.....			2	—	0	.....			2	—	0	2	—	0	2	—	0	.....			.....		
Cinnamon	.....			.....			.....			2	—	0	.....			2	—	0	2	—	0	.....		
1 per cent																								
Celery seed	2	2	0	2	2	0	2	2	0	2	1	0	2	2	0	2	2	0	2	2	0	2	2	0
Allspice	2	—	0	2	—	0	2	1	0	1	—	¾	2	1	0	1	—	¼	2	—	0	2	1	0
Peppercorn	2	2	0	2	1	0	2	2	0	2	—	0	2	—	0	2	—	0	2	2	0	2	2	0
Mustard seed	2	2	0	2	1	0	2	2	0	2	1	0	2	2	0	2	2	0	2	2	0	2	2	0
Black pepper	2	1	0	2	2	0	2	2	0	2	1	0	2	—	0	2	—	0	2	1	0	2	2	0
Cloves	1	—	½	1	—	½	1	—	½	1	—	½	1	—	¾	—	—	0	1	—	1½	2	—	0
Mace	2	2	0	2	—	0	2	2	0	2	—	0	2	—	0	2	1	0	2	2	0	2	2	0
Nutmeg	.....			.....			.....			.....			2	—	0	2	—	0	2	1	0	2	2	0
Cinnamon	2	2	0	2	1	0	2	2	0	2	—	0	2	—	0	2	—	0	2	—	0	2	2	0
Ginger	2	2	0	2	2	0	2	2	0	2	1	0	2	1	0	2	—	0	2	2	0	2	2	0
Mustard	2	2	0	2	2	0	2	2	0	2	—	0	2	2	0	2	2	0	2	2	0	2	2	0

Table 6 *(Concluded)*

5 per cent														
Celery seed	2	1	0	1	—	0	1	1	0	2	2	0	2	2	0	2	2	0	2	1	0	2	1	0
Allspice	1	—	0	1	—	0	1	—	0	1	—	0	1	—	0	1	—	0	1	—	0	1	—	0
Peppercorn	2	1	0	1	—	0	2	—	0	2	—	0	2	—	0	2	—	0	2	—	0	2	—	0
Mustard seed	2	2	0	2	2	0	1	1	0	2	—	0	2	2	0	2	2	0	1	1	0	2	1	0
Black pepper	2	2	0	2	1	0	1	—	0	2	—	0	2	—	0	2	1	0	1	—	0	2	2	0
Cloves	1	—	1	1	—	1	1	—	1	1	—	1	1	—	½	1	—	1	1	—	1	1	—	½
Mace	2	1	0	2	1	0	2	—	0	2	—	0	2	—	0	2	—	0	2	—	0	2	1	0
Nutmeg	2	—	0	2	—	0	2	—	0	2	—	0	2	—	0	2	—	0	2	—	0	2	—	0
Cinnamon	2	—	0	1	—	½	1	—	½	1	—	0	1	—	¼	1	—	¼	1	—	¼	1	—	¼
Ginger	2	1	0	1	—	0	2	—	0	2	—	0	2	—	0	2	—	0	2	—	0	2	—	0
Mustard	2	2	0	2	2	0	2	1	0	2	—	0	2	—	0	2	—	0	1	1	0	2	2	0
10 per cent spice added to nutrient agar																								
Celery seed	2	2	0	2	1	0	2	1	0	2	—	0	2	—	0	1	—	⅜	2	1	0	2	2	0
Peppercorn	2	2	0	.....			.....			.....			.....			.....			.....			.....		
Mustard seed	2	2	0	2	1	0	2	2	0	2	—	0	2	2	0	2	2	0	2	1	0	2	2	0
Pepper	2	1	0	2	—	¼	2	1	0	.....			.....			1	—	1	.....			2	1	0
Mace	2	—	0	2	—	0	.....			.....			.....			.....			.....			2	1	0
Ginger	2	1	0	.....			.....			.....			.....			.....			2	—	0	2	1	0
Mustard	2	2	0	2	—	0	2	1	0	.....			.....			.....			1	—	0	2	1	0
20 per cent spice added to nutrient agar																								
Celery seed	2	2	0	2	—	0	2	—	0	.....			.....			.....			2	—	0	2	1	0
Mace	.....			.....			.....			.....			.....			.....			.....			2	—	0
Mustard	1	—	0	.....			1	—	0	.....			.....			.....			.....			1	—	0

[1] A = growth on nutrient agar; 2 = abundant growth; 1 = moderate growth. B = growth on spiced nutrient agar; 2 = abundant growth; 1 = moderate growth; — = no growth. C = proximity of growth to spiced agar measured in inches; 1½ = 1½″; 1 = 1″; ¾ = ¾″; ½ = ½″; ⅜ = ⅜″; ¼ = ¼″; 0 = growth to the edge of spiced agar.

The data (Table 4) again show that only one organism, No. 8, was inhibited by cinnamon in a dilution of 1:100 in broth. The infusion of cloves had a greater inhibiting action than cinnamon since organism No. 7 was inhibited in a dilution of 1:200 and organism No. 8 in a 1:1,000 dilution. *Staphylococcus aureus*, an organism unusually resistant to most germicides, was inhibited by cloves in a dilution of 1:800.

BACTERIAL GROWTH ON SPICED AGAR

Preliminary work with infusions of spices in broth demonstrated that it was not possible to obtain an infusion sufficiently concentrated to gain much information by this means. A different technic was used, therefore, to test some of the more common spices and oils.

The technic used was to add different percentages by weight of the ground spices or oils of the spices to nutrient agar. This was poured into a petri dish and allowed to harden, after which the spiced agar was removed from one-half of the petri dish and nutrient agar poured into this space. The plates were incubated 24 hours to test for sterility, and if sterile they were then streaked with the eight organisms previously described. By this method it was possible to check the amount and rate of growth on the spiced agar with that on the nutrient agar. There was also diffusion of the spices from the spiced agar into the unspiced agar so that it was possible to obtain additional information as to the influence of different spices on bacterial growth by noting how close to the spiced agar the bacteria grew. The data obtained by the agar-plate method with ground spices are given (Table 6), and those obtained with the oils and a 50-per cent emulsion of the oils (Table 7). A photograph of some of the plates shows the growth of the different bacteria on the spiced and unspiced nutrient agar and the antibacterial action of the diffused spices (Fig. 1).

Our results with the antibacterial action of spices showed that cloves and cinnamon have the greatest antibacterial action on bacteria. Cloves inhibited organism No. 8 in a dilution of 1:1,000 and *Staphylococcus aureus* in a dilution of 1:800, whereas cinnamon was effective only against organism No. 8 in a 1:100 dilution. Allspice, peppercorn, and nutmeg inhibited growth in 0.5-per cent concentration, but none of the other common spices tested showed any effect until 5-per cent concentrations were used. At this concentration mace, ginger, and mustard inhibited the growth of certain bacteria used. It was also noted that there is a decided difference in the resistance of different organisms to the same spice and the same organism to

TABLE 7

Antibacterial Action of Different Percentages of Oil of Spices and a 50-Per Cent Emulsion of Oil of Spices in Nutrient Agar

Spice	Organism																							
	No. 2			No. 3			No. 7			No. 8			*Bacillus subtilis*			*Staphylococcus aureus*			*Eberthella typhosa*			*Escherichia coli*		
	A	B	C[1]	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
.1 per cent oil of spices																								
Cloves	2	—	½	2	—	½	2	—	½	2	—	½	1	—	0	1	—	0	2	—	1	2	—	½
1 per cent oil of spices																								
Caraway	1	—	0	1	1	0	1	1	0	1	—	0	2	+	0	2	1	0	2	1	0	1	1	0
Cloves	1	—	1	1	—	1	1	1	0	—	—	0	1	—	1	1	—	1½	1	—	1	1	—	½
5 per cent oil of spices																								
Caraway	1	—	1	1	—	1	1	—	1	1	—	1	1	—	1	1	—	1	1	—	0	1	—	1
Cloves	1	—	1	1	—	1	1	—	1	1	—	1	1	—	1	1	—	1½	1	—	1	1	—	1
.1 per cent oil of spices in a 50-per cent emulsion																								
Cassia	2	1	0	1	1	0	2	1	0	1	—	0	1	—	0	1	—	0	1	—	0	2	1	0
Mustard	1	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1	1	0
Allspice	2	1	0	2	1	0	1	1	0	2	1	0	2	1	0	2	1	0	2	1	0	2	1	0
1 per cent oil of spices in a 50-per cent emulsion																								
Cassia	—	—	0	—	—	0	—	—	0	—	—	0	—	—	0	—	—	0	—	—	0	—	—	—
Mustard	2	—	0	2	—	0	2	—	0	2	—	0	2	—	0	2	—	0	2	1	0	2	1	0
Allspice	—	—		1	—	½	2	—	¼	1	—	½	1	—	½	1	—	1	1	—	½	2	—	0
Ginger	2	2	0	2	—	0	2	2	0	2	—	0	2	2	0	2	1	0	2	2	0	2	2	0
Nutmeg	2	2	0	—	—	0	2	2	0	2	—	0	2	2	0	2	+	0	2	2	0	2	2	0
Celery seed	2	2	0	2	—	0	2	2	0	2	—	0	2	2	0	2	1	0	1	1	0	1	1	0
5 per cent oil of spices in a 50-per cent emulsion																								
Ginger	2	—	0	2	—	0	2	—	0	2	—	—	2	—	0	2	—	0	2	1	0	2	2	0
Nutmeg	2	—	0	2	—	0	2	—	0	2	—	—	2	—	0	1	—	0	2	1	0	2	2	0
Celery seed	2	—	0	2	—	0	2	—	0	2	—	—	2	—	0	2	—	0	2	1	0	2	2	0

[1] A = growth on nutrient agar; 2 = abundant growth; 1 = moderate growth. B = growth on spiced nutrient agar; 2 = abundant growth; 1 = moderate growth; — = no growth. C = proximity of growth to spiced agar measured in inches; 1½ = 1½″; 1 = 1″; ½ = ½″; ¼ = ¼″; 0 = growth to the edge of spiced agar.

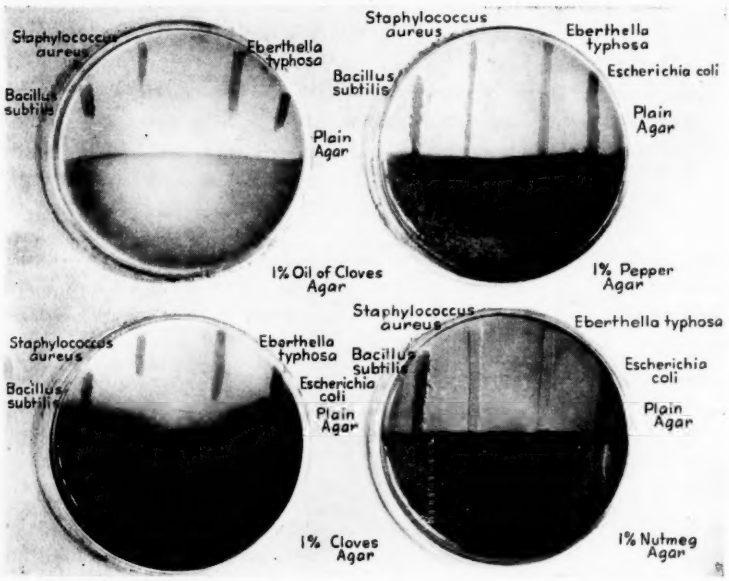

FIG. 1. Reading left to right:

Plate 1. Upper left-hand corner. Upper half of plate is plain agar. Lower half of plate is plain agar plus one per cent oil of cloves. Left to right—streak cultures of *Bacillus subtilis, Staphylococcus aureus, Eberthella typhosa* and *Escherichia coli.*

Plate 2. Upper right-hand corner. Upper half of plate is plain agar. Lower half of plate is plain agar plus one per cent ground black pepper. Left to right—streak cultures of *Bacillus subtilis, Staphylococcus aureus, Eberthella typhosa* and *Escherichia coli.*

Plate 3. Lower left-hand corner. Upper half of plate is plain agar. Lower half of plate is plain agar plus one per cent ground cloves. Left to right—streak cultures of *Bacillus subtilis, Staphylococcus aureus, Eberthella typhosa* and *Escherichia coli.*

Plate 4. Lower right-hand corner. Upper half of plate is plain agar. Lower half of plate is plain agar plus one per cent nutmeg. Left to right—streak cultures of *Bacillus subtilis, Staphylococcus aureus, Eberthella typhosa* and *Escherichia coli.*

different spices. This confirms the work of Bachmann (1916) who reached the same conclusion regarding the inhibiting action of spices on microörganisms.

One interesting observation is the susceptibility of *Staphylococcus aureus* to the action of spices. It is inhibited to a greater extent in most instances than are other bacteria which one would consider more likely to be susceptible, such as the aerobic sporulating bacteria or such bacteria as *Eberthella typhosa* or *Escherichia coli.* This is

important since it has been shown recently that *Staphylococcus aureus* is implicated in many cases of food poisoning.

Hoffman and Evans (1911) and Lahache (1919) found cinnamon, cloves, and mustard oil to be distinctly preservative in action. Grove (1918) found the oils of cloves, mustard, aniseed, and cinnamon to have a higher preserving action than any of the other spices tested. Prasad and Joshi (1929) found only cloves to have any preserving action when used by themselves. James (1931) showed that cinnamon and cloves were equally effective against *Escherichia coli* but mustard was much less effective.

The results reported here agree with those found in the literature. The oil of mustard in .1-per cent concentration in a 50-per cent emulsion gave some inhibition on nutrient agar to which it was added, and 1 per cent in a 50-per cent emulsion gave complete inhibition. The ground mustard was not effective until a 5-per cent concentration had been reached. Ground allspice was considerably more effective in preventing bacterial growth than was mustard. James (1931) stated that one sample of old mustard had no inhibiting power. The age of mustard evidently affects its antibacterial power. The diffusability of the different spices as judged by the distance of growth on nutrient agar from the spiced agar shows that cinnamon, cloves, and allspice diffuse to the greatest extent.

EXPERIMENTS WITH SPICES IN PROCESSED DILLS

After finding that many spices now being sold contained many thousands and in some instances millions of bacteria, and also finding that very few of the spices contained any bactericidal action, experiments were conducted to determine just how important a part spices may play in spoiling some of the foods to which they are added.

For our work processed dill pickles, pickled pears, and pickled peaches were used. The processed dill pickles were prepared from salt stock by freshening the pickles for 24 hours in running water and then processing them as follows:

Two gallons of pickles were made up at four different acidities, viz., .30, .48, .66, and .78 per cent respectively, with 50-grain cider vinegar. Sufficient salt was added so that the final salinity was 20 degrees salometer. To each batch was added one and one-fourth pounds of dill weed and 10 grams of spices.

Mixed spices were used which had a bacterial content of 67,000,000 bacteria per gram (Table 1). The brine which was on the dill weed used in these experiments had a bacterial count of 1,800,000 per ml. In order to secure information on the influence of the various ingre-

dients on the spoilage of the pickles five different experiments were set up as follows:

Experiment No.	Pickles	Dill weed	Spices
I	Sterile	Sterile	Not sterile
II	Not sterile	Sterile	Sterile
III	Sterile	Not sterile	Sterile
IV	Sterile	Sterile	Sterile
V	Not sterile	Not sterile	Not sterile

The various materials were sterilized by steaming them for three successive days in a steamer. Samples of the brine from the different experiments were allowed to stand for one hour after the unsterilized ingredients had been added, after which they were plated on sodium caseinate agar. The pickles were then placed in sterile quart jars, sealed air-tight, and not opened for two months or more, at which time a second bacterial analysis was made.

The results of the bacterial analyses of these experiments are given (Tables 8, 9, 10, and 11) and the physical condition of the pickles (Table 12).

TABLE 8

Bacterial Plate Count per Milliliter on Sodium Caseinate Agar of Processed Dill Pickles Preserved in .3-Per Cent Acetic Acid and 20-Degree Salometer Brine

Date	Exp. No.	Total plate count	Acid colonies	Peptonizers	Alkali and inert colonies
2-22-38	I	1,350,000	175,000	130,000	1,036,000
2-22-38	II	653,000	200,000	35,000	418,000
2-22-38	III	370,000	80,000	20,000	270,000
2-22-38	IV	0	0	0	0
2-22-38	V	1,520,000	300,000	170,000	1,050,000
5-7-38	I	250,000	15,000	1,000	234,000
5-7-38	II	400,000	20,000	0	380,000
5-7-38	III	4,800,000	800,000	0	4,000,000
5-7-38	IV	600,000	150,000	0	450,000
5-7-38	V	550,000	50,000	0	500,000

DISCUSSION OF RESULTS ON PROCESSED DILLS

The pickles processed at a low acidity, .38 per cent lactic acid (Table 8), showed slips when the spices and dill weed were not sterile (Table 12, Exp. I, III, and V), but were firm when the pickles were not sterile (Table 12, Exp. II) and when everything else was sterile (Table 12, Exp. IV). At the beginning of the experiment all contained many bacteria except when all ingredients were steri-

lized. At the end of the experiment there was a reduction except in Exp. III (Table 8), where there was about a twelve-fold increase and in Exp. IV (Table 8), which was sterile at the beginning but evidently became contaminated later.

At an acidity of .48 per cent as acetic (Table 9) there were slips and spotty pickles in the jars containing unsterile spices and where none of the ingredients were sterile (Table 12, Exp. I and V). Pickles in all the other jars were firm (Table 14, Exp. II, III, and IV).

In a processing brine of .66 per cent acetic acid (Table 10) slips were found in the jars containing the unsterile spices (Table 12, Exp. I) and spotty pickles in the jars containing the unsterile dill

TABLE 9

Bacterial Plate Count per Milliliter on Sodium Caseinate Agar of Processed Dill Pickles Preserved in .48-Per Cent Acetic Acid and 20-Degree Salometer Brine

Date	Exp. No.	Total plate count	Acid colonies	Peptonizers	Alkali and inert colonies
2-25-38	I	1,720,000	400,000	170,000	1,150,000
2-25-38	II	1,000,000	560,000	10,000	530,000
2-25-38	III	880,000	120,000	6,000	744,000
2-25-38	IV	0	0	0	0
2-25-38	V	1,400,000	320,000	180,000	900,000
5-14-38	I	250,000	150,000	10,000	90,000
5-14-38	II	44,000	15,000	0	29,000
5-14-38	III	200,000	20,000	0	180,000
5-14-38	IV	0	0	0	0
5-14-38	V	30,000	20,000	0	10,000

weed and in those where none of the ingredients were sterile (Table 12, Exp. III and V). Pickles in all the other jars were firm.

Pickles processed in brine at .78 per cent acetic acid showed slips in the jars having unsterile spices (Table 12, Exp. I), spotty pickles in jars having unsterile dill weed (Table 12, Exp. III), and very soft pickles in the jars where none of the ingredients were sterile (Table 12, Exp. V). The pickles in all the other jars were firm.

There was an increase in the bacterial content of all the brines except in Exp. I and IV (Table 12). The increase was mostly in the acid-producing colonies.

In most instances the bacterial content of the brine decreased during the period of storage. This was especially true of the peptonizing bacteria. It will be noted that the number of peptonizers ranged from 180,000 to none in the jars containing the unsterilized materials (Tables 8, 9, and 10). All the controls except one remained

TABLE 10

Bacterial Plate Count per Milliliter on Sodium Caseinate Agar of Processed Dill Pickles Preserved in .66-Per Cent Acetic Acid and 20-Degree Salometer Brine

Date	Exp. No.	Total plate count	Acid colonies	Pep-tonizers	Alkali and inert colonies
3-1-38	I	650,000	320,000	1,500	328,500
3-1-38	II	440,000	270,000	0	170,000
3-1-38	III	240,000	60,000	400	179,000
3-1-38	IV	0	0	0	0
3-1-38	V	530,000	270,000	10,000	250,000
5-17-38	I	350,000	180,000	0	170,000
5-17-38	II	30,000	20,000	0	10,000
5-17-38	III	65,000	30,000	0	35,000
5-17-38	IV	0	0	0	0
5-17-38	V	340,000	200,000	0	140,000

TABLE 11

Bacterial Plate Count per Milliliter on Sodium Caseinate Agar of Processed Dill Pickles Preserved in .78-Per Cent Acetic Acid and 20-Degree Salometer Brine

Date	Exp. No.	Total plate count	Acid colonies	Pep-tonizers	Alkali and inert colonies
3-8-38	I	100,000	2,000	0	98,000
3-8-38	II	150,000	15,000	0	135,000
3-8-38	III	6,000	2,000	0	4,000
3-8-38	IV	0	0	0	0
3-8-38	V	120,000	5,000	0	115,000
5-21-38	I	3,000	1,000	0	2,000
5-21-38	II	450,000	300,000	0	150,000
5-21-38	III	275,000	75,000	0	200,000
5-21-38	IV	0	0	0	0
5-21-38	V	375,000	175,000	0	200,000

TABLE 12

Physical Condition of Processed Dill Pickles Approximately Three Months After They Were Preserved With Different Percentages of Acetic Acid in 20-Degree Brine

Date preserved	Date examined	Acetic acid	Experiment No.				
			I	II	III	IV	V
		pct.					
2-22-38	6-4-38	.30	Slips [1]	Firm [2]	Slips	Firm	Slips
2-25-38	6-4-38	.48	Slips	Firm	Firm	Firm	Spotty
3-1-38	6-4-38	.66	Slips	Firm	Spotty [3]	Firm	Spotty
3-8-38	6-4-38	.78	Slips	Firm	Spotty	Firm	Very soft [4]

[1] Slips means that the skin readily separated from the rest of the pickle when pressure was applied to it. [2] Firm means that the pickles showed no signs of deterioration. [3] Spotty means that the pickles had areas where the skin was soft and also that there were some good pickles where there had been no change. [4] Very soft means that the deeper parenchymatous cells had been affected as well as the skin.

sterile. When there was an increase in the bacterial content during storage, the increase was due to either the acid-producing bacteria or alkali and inert group and not to the peptonizers. In only two instances did the peptonizers survive the storage period and that was at the two lower acidities, .30 and .48 per cent, in the case of the unsterile spices (Table 8, Exp. I and Table 9, Exp. I).

These experiments demonstrate that heavily contaminated materials, such as spices and dill weed, when added to freshened pickles may cause a deterioration in the pickles sufficient to cause slips or even soft pickles. This may be an unsuspected source of contamination leading to spoilage not heretofore generally recognized.

EXPERIMENTS WITH SPICES IN PICKLED PEACHES AND PEARS

This series of experiments was designed to determine the influence of sterilized and unsterilized spices on peaches and pears that had been preserved in a weak acid and sugar syrup. All the peaches and pears, except in Experiments 1 and 4, were peeled and sterilized in the syrup by heating in the autoclave at 10 pounds pressure for 20 minutes. When the spices were sterilized, they were added to the jar containing the fruit and syrup and heated with them in the autoclave. This method of sterilizing caused some breakdown of the fruit tissue, but allowance was made for this in determining the physical condition of the spiced fruits at the end of the six-months period of storage.

The mixed spices used were the same as those used in making the processed dill pickles. The bacterial plate count was 67,000,000 per gram (Table 1). The number of bacteria added to each pint of fruit from the spices was 670,000,000.

The number of bacteria was determined on sodium caseinate agar. With this medium the total count as well as the acid-producing, the peptonizing, and alkali and inert bacteria could be determined (Table 13).

The results show that the unsterilized material had the greatest number of bacteria present. The peptonizing bacteria had died out in every experiment at the end of the six-months period. It would have been interesting to see the rate at which they died, but the jars were not opened until near the end of the experiments so as to avoid contamination.

All jars that were put up with the heavily contaminated mixed spices showed signs of tissue decomposition as judged by physical examination. There was no question about this upon careful examination. Since those that were canned with the same mixed spices that had been sterilized showed no such tissue deterioration, the

TABLE 13

Experiments With Spiced Fruits

Exp. No.	Date	Plate count [1]				Bacterial conditions				Acid (calculated as acetic)	Physical changes in fruit
		Total	Acid	Peptonizers	Alkali and inert	Jars	Fruit	Syrup	Spices		
										pct.	
1 [2]	10-26-37	8,700	3,000	2,200	3,500	Not sterile	Not sterile	Not sterile	Not sterile	.84	Soft [5]
	4-16-38	10,000	5,000	0	5,000						
	5-31-38	6,000	2,500	0	3,500						
2	10-26-37	2,990	1,400	800	790	Sterile	Sterile	Sterile	Not sterile	.81	Mushy [6]
	4-16-38	15,000	8,000	0	7,000						
	5-31-38	6,000	2,500	0	3,500						
3	10-26-37	0	0	0	0	Sterile	Sterile	Sterile	Sterile	.84	No change [7]
	4-16-38	0	0	0	0						
	5-31-38	0	0	0	0						
4 [3]	10-26-37	1,140	220	380	740	Not sterile	Not sterile	Not sterile	Not sterile	.54	Mushy
	4-16-38	25,000	7,000	0	18,000						
	5-31-38	1,500	800	0	700						
5	10-26-37	1,780	230	90	1,460	Sterile	Sterile	Sterile	Not sterile	.54	Soft
	4-16-38	4,000	1,800	0	2,200						
	5-31-38	2,000	1,000	0	1,000						
6	10-26-37	0	0	0	0	Sterile	Sterile	Sterile	Sterile	.69	No change
	4-16-38	0	0	0	0						
	5-31-38	0	0	0	0						
7 [4]	10-26-37	0	0	0	0	Sterile	Sterile	Sterile	Sterile	.69	No change
	4-16-38	0	0	0	0						
	5-31-38	0	0	0	0						

[1] Plate count of whole mixed spice was 67,000,000 per gram. [2] Experiments 1, 2, 3, and 7 were done with peaches. [3] Experiments 4, 5, and 6 were done with pears. [4] Experiment 7, soluble sugar-base spices were used. [5] Soft means there was evidence of cellular changes. [6] Mushy means there was marked evidence of cellular changes. [7] No change means no cellular changes were evident.

conclusion is that sterilization inactivated or destroyed the agent or agents in the spices responsible for the breakdown of the tissues. There are two possibilities in this connection — it may have been due to enzymes or it may have been due to the bacteria present in the spices. Unfortunately, the first of these two possibilities was not anticipated at the beginning of this work so no experiments were assembled to demonstrate it. Assuming bacteria to be the cause of the tissue breakdown, what are the facts that may be marshaled to support this thesis? All samples which became soft or mushy had bacteria present while those containing no bacteria showed no change. If bacteria did cause the softening, then what group would be most likely responsible? Fabian and Johnson (1938) showed that aerobic sporulating bacteria, such as *Bacillus mesentericus fuscus,* were able to cause softening of pickles within six hours under favorable conditions. These bacteria, however, did not grow above .2 per cent acetic or .3 per cent lactic acid and 11 per cent salt, whereas the enzymes produced by them were active up to 1.1 per cent acetic and 1.05 per cent lactic acid and two per cent salt. This would explain why the peptonizing bacteria disappeared, and may or may not explain why spoilage occurred in the dill pickles and pickled fruits to which were added the unsterilized spices containing peptonizing bacteria.

Whether spoilage was caused by enzymes present in the spices or by bacterial enzymes or the bacteria themselves, one thing is certain—only sterile spices should be used in food products, such as dill pickles, pickled peaches, and pears.

SUMMARY

Samples of whole and ground spices purchased on the open market had a bacterial plate count ranging from 0 to 67,000,000 per gram.

Samples of the oils of spices, 50-per cent emulsions of spices, and spices in a sugar-soluble base were found to be sterile.

Ground cinnamon and cloves were the only spices that exhibited any inhibiting action on bacterial growth in low concentrations. Ground peppercorn and allspice showed inhibiting action in one-per cent concentrations; mustard, mace, nutmeg, and ginger in five-per cent concentrations; and celery used in 10- and 20-per cent concentrations in nutrient agar against a majority of the bacteria tested. Some bacteria grew on all concentrations of some of the spices.

There is a great difference in the resistance of different bacteria to the same spice and the same organism to different spices.

Staphylococcus aureus was more susceptible to the action of spices than many of the other bacteria tested.

The oils of spices, 50-per cent emulsions of the oils and soluble-sugar bases of the oils, were more inhibitory than the ground spices.

Unsterilized spices, when added to processed dill pickles, spiced peaches, and pears, caused a weakening in the cellular structure which led to slippery and soft pickles and to soft and mushy fruit.

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FURTHER STUDIES ON BEHAVIOR OF MICROÖRGANISMS IN FROZEN CULTIVATED BLUEBERRIES[1]

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The microbiological studies on cultivated blueberries *(Vaccinium corymbosum)* in frozen pack, as reported by Smart (1937), were lacking in many features which had been included in the original plans for this experiment. For instance, it had been planned to test the efficiency of two methods of washing the fruit and also to determine the relative reduction in microbial content of blueberries at three storage temperatures. As only one method of washing and one storage temperature were investigated in the 1935 pack, further experiments were begun in 1937 to complete these studies.

The object of this study was to determine the effects of two washing methods on the microbial content of washed berries and the reduction in the numbers of microörganisms occurring in these berries stored at —6.7°C.(20°F.) and at —17.8°C.(0°F.). Some data are given on the effect on the microbial content of holding blueberries at 7.2°C.(45°F.) for 24 hours before freezing.

MATERIALS AND METHODS

The cultivated blueberries used in this experiment were grown in the vicinity of Pemberton, New Jersey, and were purchased in the wholesale market in Washington, D. C., about 24 hours after they were picked. The berries, of an unknown variety, were large, dark blue, clean, uniform in size, finely flavored, and fairly sweet. The berries were picked over by hand to remove débris and soft, green, or withered fruit, and were divided into two lots for the washing tests.

The first lot of berries was washed by placing in a pan of water, moving them about to remove the dirt, and dipping them out to drain. The second lot was placed in a colander and water was allowed to run over them while the berries were rolled back and forth somewhat to insure maximum cleaning. After washing and draining, the berries were divided into smaller portions for the various treatments of storage temperatures, packing methods, and sirup concentrations. Twelve different types of packs involving the various treatments of storage temperatures, etc., were prepared.

[1] Food Research Division Contribution No. 402.

TABLE 1

Microbial Content of Fresh and Frozen Cultivated Blueberries Packed July 28, 1937

Pack No.	Unwashed berries	Berries washed by ordinary method	Berries washed by running water over them	Treatment			After 9 months' storage	Reduction in microbial content	
				Concentration of sirup	Holding period before freezing	Freezing and storage temperature			
	per gm.	*per gm.*	*per gm.*	*pct.*			*per gm.*	*pct.*	
100	5,000	2,250	.....	50	Frozen at once.	—17.8°C.(0°F.)	775	65.5	Average 59.7
101	5,000	.....	1,500	50	Frozen at once.	—17.8°C.(0°F.)	647	56.8	
102	5,000	.....	1,500	None	Frozen at once.	—17.8°C.(0°F.)	650	56.8	
104	5,000	.....	1,500	50	Frozen at once.	—6.7°C.(20°F.)	Less than 50	99.9+	
103	5,000	.....	1,500	50	24 hours at 7.2°C.(45°F.)	—17.8°C.(0°F.)	2,810	Increased [1]	

[1] The microbial content of these packs was increased to such an extent by holding them at 7.2°C.(45°F.) for 24 hours that even after the reduction brought about by freezing storage at —17.8°C.(0°F.) for nine months the content was nearly twice that of the fresh washed fruit.

Only air-tight packs were prepared, using R. enamel No. 2 tin cans. Twelve ounces of fruit and 200 c.c. of sirup filled the cans so that there was no bulging when the contents were frozen. The packing was done on two different days, using a crate of berries each time.

While trying to vary the conditions of packing so as to test adequately their effect on the reduction of the microbial content of frozen blueberries, a satisfactory variation in the method of packing this fruit was found. This method, which has not been seen in the literature, consisted of washing and drying the fruit and freezing it on trays at —17.8°C.(0°F.) and then placing the individually frozen berries into cans and covering them with cold sirup, 30°C.(86°F.). The cans were then sealed and placed in the freezing room before any thawing could take place. The usual method is to put the berries in the container, cover them with sirup, seal, and freeze.

Samples of blueberries were taken and bacteriological examinations were made at all stages during the washing, packing, freezing, and storage procedures. Methods used in making these examinations have been described by Smart (1937).

EXPERIMENTAL DATA AND DISCUSSION

The data collected during these studies are considered in two sections (Tables 1 and 2) as the fruit handled on the two days was found to be of different quality in regard to microbial content and was given different treatment as to packing methods.

A study of the types of bacteria, yeasts, and molds in both the fresh and frozen fruit was made, but the conditions were found to be similar to those already given by Smart (1937) for the 1935 pack and will not be repeated.

Dip washing the berries (Table 1) definitely appears to have lowered the bacterial content, while washing in a colander and rotating had a much more decided effect in reducing the numbers of bacteria present in the berries. After nine months' storage at —17.8°C. (0°F.) the fruit washed by the first method had a higher microbial content than fruit washed by the second method. While the difference in microbial content was not great in the frozen fruit of the two lots there was enough difference to show that a high microbial content of frozen fruit may be an indication of inefficient methods of washing the raw product.

Moon, Smart, and Caldwell (1936) have reported on the proper degree of maturity, the choice of varieties, the amount and concentration of sirup to be used, and other factors which are essential to the production of satisfactory frozen blueberries. Diehl, Pentzer,

TABLE 2

Microbial Content of Fresh and Frozen Cultivated Blueberries Packed August 2, 1937

Pack No.	Washed by running water over berries	Treatment			After 9 months' storage temperature	Reduction by freezing storage	
		Concentration of sirup	Method of freezing	Freezing and storage temperature			
	per gm.	*pct.*			*per gm.*	*pct.*	
106	3,400	50	Berries frozen in sirup.	—17.8°C.(0°F.)	1,300	61.8	
107	3,400	40	Berries frozen in sirup.	—17.8°C.(0°F.)	930	72.7	Average 60.3
108	3,400	30	Berries frozen in sirup.	—17.8°C.(0°F.)	1,820	46.5	
109	3,400	50	Berries frozen separately on trays, then sirup added.	—17.8°C.(0°F.)	1,440	57.7	
110	3,400	40	Berries frozen separately on trays, then sirup added.	—17.8°C.(0°F.)	1,210	64.5	Average 57.7
111	3,400	30	Berries frozen separately on trays, then sirup added.	—17.8°C.(0°F.)	1,670	50.9	

Berry, and Asbury (1934) have given the same information for the correct handling of frozen blueberries in the Pacific Northwest. While the experimental work done in 1937, now being reported, was not done for the purpose of determining proper packing methods, the quality of the berries prepared by covering them with 40 or 50 per cent sirup either before or after the berries were frozen at 0°F. was said to be excellent by many competent judges in the U. S. Department of Agriculture. Most of the judges preferred the berries which had been frozen before the sirup was added.

The berries frozen and stored at 0°F. in 1937 had no better flavor, color, odor, or texture than those frozen and stored at —9.4°C.

TABLE 3

Effect of Storage Temperature on Microbial Content of Frozen Cultivated Blueberries

Year	Description of packs	Freezing and storage temperatures for 9 months	Reduction in microbial content during storage
			pct.
1937 (Table 1)	Frozen in 50% sirup in sealed tin cans.	—6.7°C.(20°F.)	99.9+
1935 (Smart, 1937)	Frozen in 50% sirup in sealed tin cans.	—9.4°C.(15°F.)	99.6
1937 (Table 1)	Frozen in 50% sirup in sealed tin cans.	—17.8°C.(0°F.)	59.7

(15°F.) in 1935, according to the opinion of the judges who tasted both lots. Berries stored at —6.7°C.(20°F.) were shriveled, off-color, and entirely unfit for food.

It was noted (Table 1) that the reduction in microbial content of blueberries during the nine months' storage at 0°F. amounted to 59.7 per cent, while at 20°F. for the same period the content was reduced 99.9+ per cent. The fruit held at 7.2°C.(45°F.) for 24 hours before it was frozen and stored at 0°F. for nine months had a microbial content higher than the fresh washed fruit. Considering the fact that freezing storage at 0°F. reduced the counts in this type of pack as well as in those frozen immediately, it becomes evident that the numbers of microörganisms had increased enormously during the holding period of 24 hours at 45°F. and that this practice is not to be recommended.

The results (Table 1) show that in two instances high microbial content in frozen blueberries may be an indication of faulty handling methods, such as inefficient washing or holding the packed fruit too long before freezing it. On the other hand, a product with a very low microbial count (berries stored at 20°F. for nine months) was found to be unfit for food. Berry (1934) made this observation working with strawberries and raspberries stored at 20°F. These findings make it obvious that the microbial content of frozen blueberries is one of many factors which must be considered in judging the quality of this product.

It was noted (Table 2) that the fruit frozen before the sirup was added had a slightly lower microbial count than when the berries were frozen in the sirup, but the difference was too slight to be significant.

SUMMARY

It has been shown that a high microbial content of frozen blueberries may be an indication of inefficient methods of washing the raw fruit.

Freezing and storage of blueberries for nine months at 0°F. resulted in a reduction of the microbial content amounting to 59.7 per cent, while storage at 20°F. for the same period resulted in 99.9+ per cent reduction.

Quality was preserved in blueberries when they were frozen and stored at 0°F. for nine months (also at 15°F., Smart, 1937), but the berries were not edible after storage at 20°F. for the same period.

Holding blueberries, immersed in sirup, for 24 hours at 45°F. before freezing them resulted in a high microbial content as compared with those placed in freezing storage as soon as they were packed.

The results of these experiments indicate that while bacteriological examination of frozen fruit may be relied upon to give information as to the preparation and handling of fruit, it should not be the sole index in judging quality.

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MICROBIOLOGICAL STUDIES[1] ON COMMERCIAL PACKS OF FROZEN FRUITS AND VEGETABLES[2]

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References to the microbial content of commercially packed, frozen fruits and vegetables are difficult to find in the literature. Brown (1933) has published data derived from a study of commercially packed, frozen vegetables to determine the keeping qualities of these packs as compared with fresh vegetables.

The purpose of the investigation now being reported was to study the numbers and types of microörganisms present in commercially frozen fruits and vegetables, and with such results as an index to determine the relative quality of such foods from 1929 to 1936, or during the years they were being presented to the retail trade.

Many of the samples of commercially packed, frozen fruits and vegetables used in this experiment were sent to the U. S. Department of Agriculture laboratories for examination, while others were purchased in the open market. Samples of frozen fruits and vegetables packed in all sizes and types of containers used by the industry and collected from all parts of the United States where such products are prepared, have been included in these studies.

LITERATURE REVIEW

While reports on the bacteriological examination of commercially packed fruits and vegetables are difficult to find in the literature, the opposite is true of papers dealing with the survival of microörganisms at low temperatures. Most of this work, which has been reviewed by Wallace and Tanner (1933) and Berry and Magoon (1934), has been of interest to investigators dealing with the microbiology of frozen fruits and vegetables.

More recently Prescott and Tanner (1938) have reviewed work published on the effect of low-temperature preservation on the microbial content of foods, both from the point of view of commercial production and public health.

[1] These studies were begun in May, 1929, while the writer was a member of the Bureau of Plant Industry, U. S. Department of Agriculture, and were continued after her transfer to the Bureau of Chemistry and Soils in 1935.

[2] Food Research Division Contribution No. 403.

TABLE 1

Microbial Content of Commercially Frozen Fruits and Vegetables[1]

Product[2]	Year examined							
	1929	1930	1931	1932	1933	1934	1935	1936
	per gm.	*per gm.*	*per gm.*	*per gm.*	*per gm.*	*per gm.*	*per gm.*	*per gm.*
Blackberries	72	80	283	150	.....	.....	.....	20
Blueberries	.....	.....	.....	.....	5,000	.....	1,500	.....
Cherries	3,000	.....	.....	160	.....	3,500	2,000	300
Raspberries (red)	1,860	1,160	379	200	.....	.....	.....	200
Strawberries	20,000	55,000	300,000	1,500	95,000	1,200	360	150
Sweet corn	.....	.....	205,000	200,000	19,200,000	150,000	1,500,000	30,000
Beans (green and wax)	.....	.....	.....	.....	486,000	500,000	15,000	47,000
Beans, lima	.....	.....	153,000,000	1,750,000	512,000,000	5,000,000	170,000	18,000
Mushrooms	.....	.....	10,500	5,000	500,000	.....	.....	.....
Peas	.....	.....	50,000,000	300,000	80,000,000	2,500,000	1,000,000	200,000
Spinach	.....	.....	3,000,000	1,250,000	136,000	50,000	.....	60,000

[1] Numbers of samples examined range from three to 50. [2] Freezing and storage temperature, length of storage, or other handling methods are not known.

EXPERIMENTAL PROCEDURE

The frozen material to be examined was partly defrosted before the containers were opened. This process was expedited when the containers were tin, by placing them in cold water. At the time samples were removed for plating, the material was defrosted sufficiently to allow thorough mixing with a sterile spoon, but ice was still evident in the individual pieces of food. When fruits and vegetables to be examined were packed in containers so large that thorough mixing was impossible, samples were removed from many parts of the mass and these counts averaged for the microbial content.

One-hundred-gram samples were weighed out into sterile jars, mashed with a sterile spoon, and mixed well with 100 c.c. of added sterile tap water. Following thorough mixing, dilutions were made into a series of nine c.c. water blanks and plates were poured. Beef infusion agar (pH 7) was used to determine the number of bacteria while wort agar [3] (pH 4.5, approx.) supported a good growth of yeasts and molds and was used to determine the numbers of these types present.

The beef agar plates were incubated for two days at 30°C. (86°F.), while the wort agar plates were allowed five days' incubation after which colonies were counted to determine the microbial content per gram of material. Individual colonies were examined microscopically and culturally to determine the bacterial flora of each sample.

MICROBIAL CONTENT OF COMMERCIALLY FROZEN FRUITS AND VEGETABLES

The microbial content (Table 1) of commercially frozen fruits and vegetables as collected from 1929 to 1936 inclusive, has been summarized and studied. A wide variation in microbial content of frozen fruits and vegetables from year to year is to be expected owing to geographical as well as seasonal climatic factors. The microbial content of these products may have been affected also by variations in the processes of packing, storing, and shipping.

Frozen fruits examined were found to contain fewer microörganisms per gram than frozen vegetables. The high acidity of most fruits and the sugar in which they were packed, acted as inhibiting factors to the growth of bacteria.

The commercially packed, frozen fruits and vegetables examined in 1935 and 1936 do not have the extremely high microbial counts

[3] Formula for wort agar:

Hop-flavored malt syrup	100 grams
Distilled water	1,000 c.c.
Agar	15 grams.

that were found in some of these products prior to that year. This record may indicate that these products were more correctly packed, stored, and shipped in 1935 and 1936 than they were in former years.

TYPES OF MICROÖRGANISMS FREQUENTLY ISOLATED

The types of microörganisms most frequently isolated from commercially frozen fruits and vegetables were the common soil types which have always been considered to have no health significance

TABLE 2

Types of Microörganisms Repeatedly Isolated From Commercially Frozen Fruits and Vegetables

Fruits	Vegetables
Bacteria:	Bacteria:
Achromobacter butyri Bergey, *et al.*	*Achromobacter pellucidum* Harrison
Bacillus mycoides Flügge	*Bacillus mycoides* Flügge
Bacillus aterrimus Lehmann u. Neumann	*Flavobacterium aurescens* (Ravenel) Bergey, *et al.*
Pseudomonas syncyanea (Ehrenberg) Migula	*Lactobacillus* sp.
Spirillum volutans (Ehrenberg)	*Pseudomonas fluorescens* (Ehrenberg) Migula
Yeasts:	*Sarcina sp.*
Saccharomyces unisporus Jörgensen	*Sarcina subflava* Ravenel
Saccharomyces exiguus Reess-Hansen	Yeasts:
Molds:	Torula sp.
Aspergillus sp.	Molds:
Mucor sp.	Dematium sp.
Oidium sp.	Penicillium sp.
Penicillium sp.	Rhizopus sp.
Rhizopus sp.	

but which will cause spoilage of the food provided it is not used promptly after defrosting.

Bacteria belonging to the genera *Sarcina*, *Flavobacterium*, and *Bacillus* (Table 2) were the types most frequently isolated from commercially packed frozen vegetables. Lochhead and Jones (1938) have found coccus forms to be numerous in their experimental packs of frozen vegetables. Berry (1933) found Lactobacilli abundant in frozen peas.

EFFECT OF HOLDING DEFROSTED FROZEN FRUITS AND VEGETABLES

Three packages each of commercially packed, frozen blackberries, raspberries, strawberries, corn, peas, and spinach were allowed to defrost and remain at room temperature, 30°C.(86°F.), for 24 hours

to determine the types of microörganisms that are responsible for causing spoilage in these products. It was found that defrosted vegetables became unfit for food in 24 hours at 30°C., while the fruits, aside from being softened in texture, were still edible.

Most of this difference in the spoilage rate is due to the difference in acidity. The high acidity of the fruits inhibits the growth of bacteria, while the pH range of most vegetable products is the optimum range for the development of many bacterial forms. Freezing and subsequent thawing so changes the physical structure of plant tissues that they become more readily attacked and invaded by microörganisms than do fresh fruits and vegetables.

TABLE 3

Effect on Microbial Content of Holding Defrosted Fruits and Vegetables[1] *for 24 Hours at 30°C.(86°F.)*

Product	Content of products as removed from storage		Content of products held 24 hrs. at 30°C.	
	Beef agar (bacteria)	Malt agar (yeasts and molds)	Beef agar (bacteria)	Malt agar (yeasts and molds)
	per gm.	*per gm.*	*per gm.*	*per gm.*
Blackberries	51	8	270	20
Raspberries	212	66	208	0
Strawberries	95	73	271	0
Corn	83,000	100	396,000,000	0
Peas	307,000	250	544,000,000	0
Spinach	57,000	200	130,000,000	0

[1] All products in non-air-tight, waxed-paper containers.

In some of the fruit examined bacterial counts were slightly higher at the end of the 24-hour period (Table 3) but their growth in the fruit did not continue. The types bringing about spoilage in the fruit examined were yeasts and molds which started to develop soon after 24 hours at 30°C.

Sarcina and other coccus forms were found in abundance on all plates poured from the spoiled vegetables. Other types, such as yeasts and surface mold, developed later.

SUMMARY

A comparison of the microbial content of commercially frozen fruits and vegetables over a period of years shows that extremely high counts in these products were less common in 1935 and 1936 than they were prior to these years.

The types of microörganisms most frequently isolated from commercially frozen fruits and vegetables were the common soil types

which have always been considered to be without health significance, but which will cause spoilage of the food provided it is not used promptly after defrosting.

Defrosted, commercially packed vegetables were found to spoil much faster than similarly treated fruits when both were held at 30°C. for 24 hours.

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ADHESION OF POTATO-TISSUE CELLS AS INFLUENCED BY PECTIC SOLVENTS AND PRECIPITANTS[1]

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(Received for publication November 25, 1938)

In a previous paper a method was described whereby the extent of weakening of the intercellular cementing material of potato-tuber tissue and the extent of decrease in cell adhesion could be followed quantitatively by tensile-strength measurements, Personius and Sharp (1938). As would be expected, heat was found to bring about a weakening of the cementing material. The decrease in cell adhesion apparently was not dependent upon starch gelatinization.

The behavior of potato tissue on heating is in agreement with the idea that the intercellular material of the tissue is pectic in nature, and during cooking this material is progressively weakened until the tissue cells can be separated readily. At this point the potato is sufficiently softened to be considered done. If this idea is correct, it should be possible to produce similar softening by chemical means. Experiments demonstrating that such is the case are presented in this paper.

It is generally accepted that the intercellular material of plant tissue is composed of pectic substances. Norman (1937), in his recent review on cellulose and related cell-wall compounds, states that the middle lamella of plant tissue is believed to be insoluble calcium pectate while the cell-wall substances are protopectin and soluble pectin. The protopectin is always found closely associated with other cell-wall constituents, especially cellulose. Protopectin can be removed from plant tissue by treatment with hot dilute acids and by hot ammonium oxalate, $(NH_4)_2C_2O_4$. Calcium pectate is also soluble in ammonium oxalate. Candlin and Schryver (1928) state that any salt which, like ammonium oxalate, yields by double decomposition calcium compounds which are insoluble in water, may be used to extract pectin from tissue. Fabian and Johnson (1938), studying the softening of pickles, found that pickles preserved in strong acid (2.2 per cent HCl) become soft owing to acid hydrolysis

[1] From a thesis presented by Catherine Personius to the Graduate School of Cornell University in partial fulfillment of the requirements for the degree of Doctor of Philosophy, 1937.

of the insoluble protopectin of the cell wall to form a soluble pectin. They also reported slight hydrolysis with similar concentrations of acetic and lactic acids.

That the intercellular cementing material of potato tissue is pectic in nature is evidenced by the fact that potato tissue has been used in testing for the presence of protopectinase, Davison and Willaman (1927). The test depends upon the ability of this enzyme to hydrolyze or dissolve the pectic lamella between the tissue cells with the resultant separation of the cells from each other.

EXPERIMENTAL PROCEDURE

If the intercellular material of potato-tuber tissue is pectic in nature, one would expect that soaking sections of tubers in solutions of acids or other pectic solvents would be effective in decreasing the cell adhesion of the tissue.

The potatoes used in these tests were mature tubers of the Smooth Rural variety which had been stored for at least four months at 4.4°C.(40°F.). The decrease in cell adhesion was measured by determining the tensile strength, using the method previously described by Personius and Sharp (1938). Comparable samples of potato tissue, either as slices approximately .8 cm. thick or as dumb-bell-shaped sections ready for testing, were placed in beakers containing the test solutions. These were held in a thermostatically controlled electric oven at 65°C.(149°F.) for the duration of the treatment. A temperature of 65°C. was chosen since preliminary tests with ammonium oxalate and sodium fluoride solutions indicated that at lower temperatures changes were too slow to be practical. Appreciable change was noted, however, in tissue held in oxalate solution at 55°C.(131°F.) for 20 hours. Since heat alone had been found to decrease cell adhesion, control tests using water or potato juice[2] were run parallel with the chemical tests. At regular intervals samples were removed for tensile-strength measurements. Since the effect of acidity was also under investigation at the time of making tensile-strength measurements, the pH of each solution was determined electrometrically using a quinhydrone electrode.

ACID SOLUTIONS

After several preliminary experiments with other acids and buffer solutions, it was decided to test the effect of acid on cell adhesion

[2] Potato juice was prepared by putting peeled potatoes through a medium-fine meat grinder, pressing juice from the ground tissue, and centrifuging the juice to remove the suspended starch. The starch-free liquid was heated to 80°C. (176°F.) to coagulate the protein and any other heat-coaguable material, then filtered.

by immersing tuber sections in dilute lactic acid solutions of various pH values.

The effect of solutions of lactic acid on the tensile strength of potato tissue is shown (Fig 1). Extensive decrease in cell adhesion resulted in all solutions having a pH of 3 or less. The tensile-strength values after 25 hours were as low as those characteristically obtained with cooked tubers, Personius and Sharp (1938). With solutions having higher pH values, weakening of the intercellular

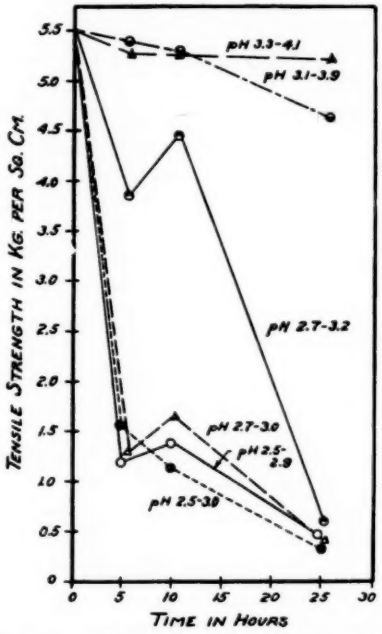

FIG. 1. Changes in the tensile strength of potato tissue after holding at 65°C. (149°F.) in lactic acid solutions of different pH values for periods of time up to 25 hours.

material was not extensive even after 25 hours. Various workers have recommended the use of acid solutions for extracting pectic substances from plant tissue. Calculation of the probable pH values of these various solutions shows them to be below pH 3. This indicates that acid hydrolysis or solution of the insoluble protopectin of plant tissues is not very rapid at higher pH ranges.

SALT SOLUTIONS

The effectiveness of a number of salt solutions in decreasing cell adhesion was found to agree in general with their ability to function

as solvents for pectic substances. Results of typical experiments are presented (Fig. 2); all solutions used were .2 normal except as follows: .08 normal citrate, fluoride, and oxalate solutions.

Chemicals capable of removing calcium, namely ammonium oxalate, sodium citrate, and sodium fluoride, were all very effective in decreasing the cell adhesion of potato tissue. The recognized pectic solvent, ammonium oxalate, was most efficient; in 25 hours the tensile

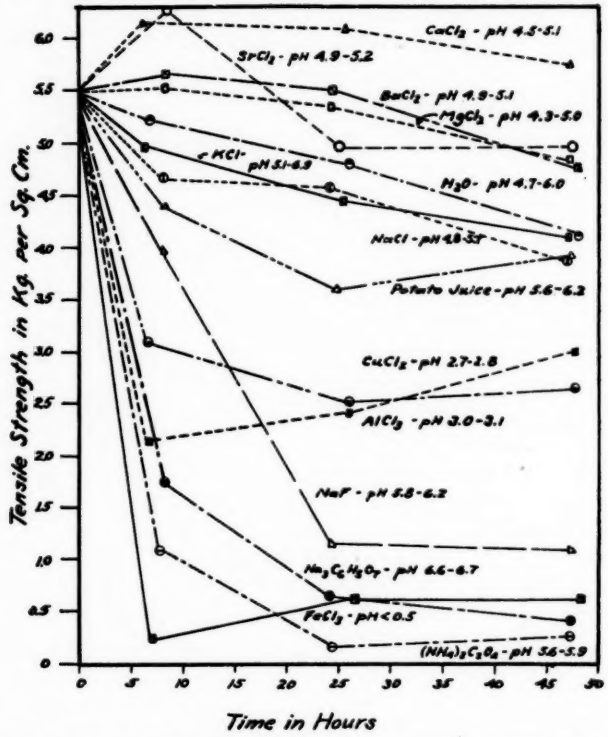
Tensile Strength in Kg per Sq Cm.
Time in Hours
$CaCl_2$ - pH 4.5-5.1
$SrCl_2$ - pH 4.9-5.2
$BaCl_2$ - pH 4.9-5.1
$MgCl_2$ - pH 4.3-5.0
HCl - pH 5.1-6.3
H_2O - pH 4.7-6.0
NaCl - pH 4.8-5.5
Potato Juice - pH 5.6-6.2
$CuCl_2$ - pH 2.7-2.8
$AlCl_3$ - pH 3.0-3.1
NaF - pH 5.8-6.2
$Na_3C_6H_5O_7$ - pH 6.6-6.7
$FeCl_3$ - pH < 0.5
$(NH_4)_2C_2O_4$ - pH 5.6-5.9

FIG. 2. Changes in the tensile strength of potato tissue after holding at 65°C. (149°F.) in various salt solutions for periods of time up to 48 hours.

strength had decreased sufficiently so that the tissue cells separated easily and the tissue could be readily mashed.

In the control tests where slices of potato tissue were held in water and in potato juice, decrease in cell adhesion was slight, indicating that the decided decrease in cell adhesion noted with the oxalate, fluoride, and citrate solutions and with the acid solutions of low pH was dependent upon the action of the chemicals used and was not the result of heat alone. Tensile-strength values of about

the same magnitude were obtained with water and with potato juice, indicating that the observed decrease in cell adhesion was not the result of a leaching effect.

These results evidence the pectic nature of the intercellular cementing material of potato tissue and support the idea that "doneness" of cooked potato is attained when this material has been weakened to such a point that the tissue cells can be separated readily.

Results obtained in the experiments with solutions having a common anion but different cations, indicate that the cation environment of potato tissue is important. The presence of the divalent cations—barium, calcium, magnesium, and strontium—was found to retard or prevent any decrease in cell adhesion of tissue held at 65°C. (149°F.). In the presence of divalent copper, however, the tensile strength of the tissue was significantly lowered. The apparently inconsistent results obtained with copper can be reasonably explained if we consider the pH values of the various solutions. The cupric chloride solution had a pH below 3, while solutions containing the other divalent cations had pH values of 4.3 or above. It has already been pointed out that an acidity of pH 3 or lower causes a decrease in the cell adhesion of potato tissue.

The decrease in tensile strength brought about by cupric chloride solution of pH 2.7 to 2.8 was not as great as the decrease obtained with a lactic acid solution of pH 2.7 to 3. This suggests that the action of the acid in decreasing cell adhesion may have been mitigated by a counter tendency of the divalent copper ion to prevent removal of calcium from the pectate or to form insoluble copper salts of pectin. This idea is supported by results obtained in tests using solutions of barium, calcium, magnesium, and strontium chlorides which had been adjusted to pH values below 3 by addition of lactic acid. In these experiments there was an appreciable decrease in cell adhesion in all cases.

It was expected that the presence of trivalent cations would produce results comparable to those obtained with divalent cations. Actually both aluminum and ferric chlorides brought about a definite decrease in cell adhesion (Fig. 2). Again these unanticipated results can be reasonably explained as due to the acidity of the solutions. Attempts to increase the pH of these solutions to approximately 4 and thus test the effect of trivalent cations in a less-acid medium were not successful.

Tissue held in solutions of the monovalent cations, sodium and potassium, showed a decrease in cell adhesion similar to that obtained in the control tests. No effect would be expected from the cation in this case since these monovalent ions should not interfere with the

removal of calcium and since both sodium and potassium pectins are soluble.

REVERSIBILITY OF EFFECT OF SALTS ON CELL ADHESION

If the removal of calcium from its combination with the intercellular cementing material of tissue or the formation of soluble from insoluble pectic substances is responsible for the observed decrease in cell adhesion after appropriate chemical or heat treatment, it seems possible that the reaction might be reversed; that is, cell adhesion might subsequently be increased, by treatment with divalent cations, such as calcium. The reversibility of the process

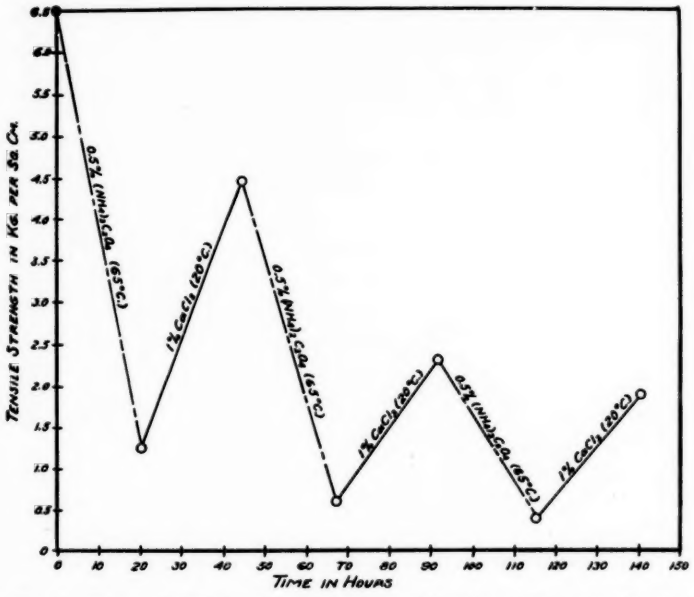

FIG. 3. Changes in the tensile strength of potato tissue after successive treatments with ammonium oxalate at 65°C.(149°F.) followed by calcium chloride at 20°C. (68°F.).

is definitely indicated (Fig. 3 and 4). Tissue, the cell adhesion of which had been reduced by chemical treatment to values characteristic of cooked potato, when treated with calcium chloride solutions regained cell-adhesive properties approaching those of natural raw-tuber tissue.

DISCUSSION

The effectiveness of calcium ions and other cations of similar properties in retarding the cell loosening which would be expected

to accompany heating, is in agreement with the view that decrease in cell adhesion of potato tissue results upon the removal of calcium from the intercellular material or upon the hydrolysis of protopectin to soluble pectins. Further support is found in results of tests in which cell adhesion of tissue was first decreased by appropriate chemical or heat treatments, and subsequently increased by holding in solutions of calcium chloride (Fig. 3 and 4). The latter treatment supposedly replaced the calcium or converted soluble pectins into an insoluble calcium pectin.

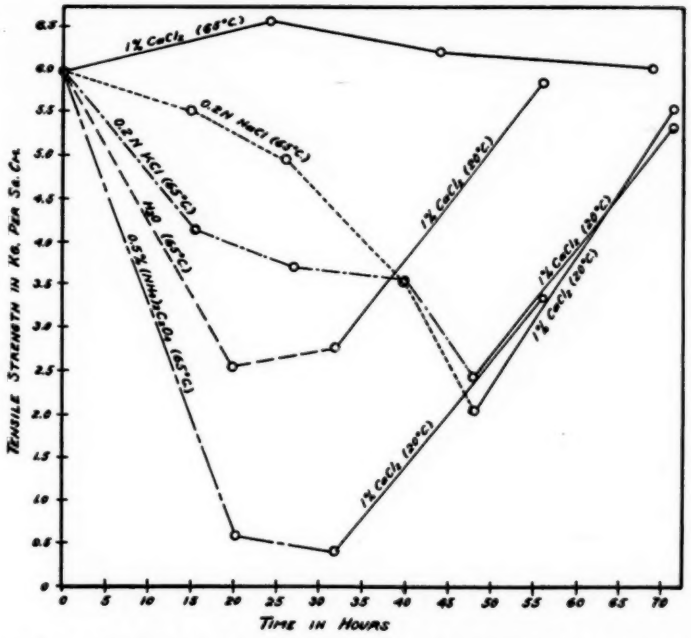

FIG. 4. Changes in the tensile strength of potato tissue held 32 to 48 hours in water or in various salt solutions at 65°C.(149°F.), then transferred to calcium chloride at 20°C.(68°F.).

These results have not been duplicated using new or immature potatoes or using potatoes stored at temperatures other than 4.4°C. (40°F.) or for periods shorter than four months. Tests are now in progress investigating the influence of some of these factors on the response of potato tissue to the various chemical treatments. Preliminary tests indicate that it takes appreciably longer to reduce, by means of ammonium oxalate solutions, the tensile strength of potato tissue from new potatoes to a value of about .5 kg. per sq.

cm. than is required for the tissue of tubers which have been in storage for several months. This is probably indication of changes in the nature or amount of the pectic constituents of the potato tuber during maturation and storage.

The part played by the cation environment of potato tissue in regulating the extent of decrease in cell adhesion which results when the tissue is held at 65°C.(149°F.), raises questions as to the importance of the cation content of the tissue itself and of any liquid medium surrounding it in determining the softening of the tissue obtained in ordinary cooking processes.

When cell adhesion of potato tissue has been decreased by chemical means until the tissue can be easily mashed, the product does not look like cooked potato. Microscopically, the outstanding difference between chemically treated and cooked tissue is that the starch of the tissue has not been gelatinized by the chemical treatment. This suggests the possibility of using a second chemical to gelatinize the starch and so by chemical means simulate cooking. Products indistinguishable in appearance from cooked potato have been obtained by appropriate chemical treatment. These experiments will be reported in detail later.

CONCLUSIONS

Using tubers which had been in storage at 4.4°C.(40°F.) for at least four months, it was possible to bring about extensive decrease in cell adhesion of potato tissue by holding at 65°C.(149°F.) in solutions of ammonium oxalate, sodium citrate, or sodium fluoride and in lactic acid solutions having a pH of 3 or less. Holding in water, potato juice, or .2 N solutions of sodium or potassium chloride resulted in definite but not extensive decrease in cell adhesion; while with .2 N solutions of barium, calcium, magnesium, or strontium chlorides little or no decrease in cell adhesion was obtained. Holding in .2 N solutions of aluminum, cupric, or ferric chlorides resulted in a decided decrease in cell adhesion probably due to the acidity (pH 3 or lower) of the solutions rather than to any effect of the cations present.

Tissue in which cell adhesion had been decreased by appropriate heat or chemical treatment, showed an increase in tensile strength after being held in a solution of calcium chloride.

The decrease in cell adhesion resulting from weakening of the intercellular cementing material of potato tissue can be reasonably explained by assuming that these changes consist of the removal of calcium from calcium pectate by means of such chemicals as ammonium oxalate, sodium citrate, and sodium fluoride and of the hy-

drolysis of insoluble protopectin to soluble pectins. The hydrolysis is favored by heat and by an acidity of pH 3 or lower. Apparently in the presence of such cations as barium, calcium, magnesium, and strontium any soluble pectins formed are converted to insoluble pectin salts thus counteracting the usual effects of hydrolysis.

These experiments are believed to indicate that the intercellular material of potato tissue is pectic in nature and that the softening of tissue characteristically obtained during cooking is the result of an extensive decrease in cell adhesion which accompanies the weakening of the intercellular cementing substance.

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VITAMIN C IN VEGETABLES. X. SNAP BEANS[1]

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Snap beans have been generally considered to be a rather poor source of vitamin C. The vitamin analyses reported in the literature, which has been well summarized by Daniel and Munsell (1937), are not in good agreement. In previous papers of this series, it has been shown that in many cases such discrepancies could be attributed to real differences between varieties, degrees of maturity, etc. But in the case of snap beans the variations are so unusually large as to suggest a serious source of error in the analyses. Accordingly, in this investigation particular care was taken first to establish the validity of the analytical methods for this vegetable. Then the influence of variety, maturity, freshness, soil type, and fertilizer treatment upon the vitamin C content of snap beans was determined.

EXPERIMENTAL PROCEDURE

Analytical Methods: The Tillmans method (1932) as modified by Bessey and King (1933) and by Mack and Tressler (1937) was used. While this method may give high results if any reducing material other than ascorbic acid is present in the vegetable extract, that possibility is precluded in our experiments by the close agreement obtained with the specific biological assay method.

The procedure used in the curative type assay has been described in detail by Tressler, Mack, and King (1936). A summary of the assay data (Table 1) indicates reasonably good agreement between the titration values and the physiological values. The feeding levels used are particularly satisfactory for this type of assay because the animals are kept in a zone of deficiency where they are very sensitive to relatively small differences in vitamin intake. The lack of agreement between the weight changes on the .25 mg. per day feeding level is probably caused by the incidence of secondary factors, such as poor appetite, intestinal disturbances, and infections, accom-

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panying the more scorbutic condition. In this case the scurvy score is considered to be more specific for quantitative interpretation.

It should be pointed out that biological assay methods will give low results if the beans are not promptly consumed by the animals. At room temperature atmospheric oxidation of the ascorbic acid in the tissues of this vegetable is very rapid. In our experiments this source of error was practically eliminated by removing all uneaten food from the animal cages within one hour after feeding. Animals which consistently refused to eat their food promptly were excluded from the assay.

The rapidity with which ascorbic acid is lost from bean tissue makes the freshness of the vegetable an important consideration.

TABLE 1

Biological Assay of Kentucky Wonder Snap Beans

	Number of animals	Feeding level of vitamin per day	Average initial weight	Average final weight	Average net gain	Average scurvy score
		mg.	*gm.*	*gm.*	*gm.*	
Kentucky beans, raw	8	.50	355	366	11	3.1
Vitamin solution	8	.50	354	402	48	3.3
Kentucky beans, raw	10	.25	354	313	—41	6.9
Vitamin solution	6	.25	377	264	—113	7.0
Negative control	6	.00	364	238	—135	17.0

Our beans were grown on the nearby Experiment Station farm, and all the chemical analyses were completed within a few hours after harvesting. The beans reserved for biological assay were frozen and kept over solid carbon dioxide. No changes could be detected during the assay period.

The sampling procedure should be carefully considered because ascorbic acid is so unevenly distributed throughout the bean pod. Different pods, even when grown on the same plant, often contain varying amounts of ascorbic acid. To obtain a representative sample, two- to three-gram sections from five to ten different pods were combined to make up 20 grams of material. It is rather important not to include more than five grams from any one pod. The small sections should be selected so that every part of the bean pod is proportionately represented in the sample.

Each sample was covered with 40 ml. of an acid mixture containing 10 per cent sulfuric and two per cent metaphosphoric acids. After grinding with sand, the solid material was separated and washed centrifugally with three successive 20-ml. portions of the

acid mixture. The extract and washing were combined and made up to 100 ml. from which aliquots were taken for titration.

Snap beans, particularly in the immature stage, contain very active oxidizing agents. In our experiments the procedure of Mack and Tressler (1937) practically prevented oxidation of ascorbic acid during the extraction, but in order to detect an occasionally significant quantity of dehydroascorbic acid, each extract was treated with H_2S before titration.

TABLE 2

Influence of Variety on Ascorbic Acid Content of Snap Beans

Variety	Date of sampling	Ascorbic acid content
		mg. per gm.
Season of 1935		
Tendergreen	8/ 6	.24
Kidney Wax	8/11	.19
Ideal Market	8/14	.20
Kentucky Wonder	8/25	.19
Kentucky Wonder Wax	8/29	.19
Refugee	9/ 5	.18
New McCaslin	9/ 7	.18
General Cornfield	9/ 9	.14
Blue Lake	9/18	.11
Georgian	10/ 9	.09
Season of 1936		
Refugee	8/31	.16
Season of 1937		
Red Kidney	8/25	.28
White Pea	8/25	.22
U. S. No. 5	8/25	.19
Refugee	8/ 2	.21
	8/ 9	.19
	8/18	.18
	9/ 5	.18

Relation Between Variety and Ascorbic Acid Content: Thirteen varieties of beans were grown on Ontario clay loam soil and sampled at approximately the same stage of maturity. Each result represents the mean of three and four separate determinations (Table 2).

The Red Kidney and White Pea varieties are ordinarily harvested as seed beans, but sometimes they are eaten at the green snap-bean stage of maturity. It is interesting to note that they rank above most of the regular snap-bean varieties in ascorbic acid content. This is probably due to the fact that they have well-filled seed pods. It will be shown later that the seeds are much richer in vitamin C than the containing pods.

Influence of Maturity and Its Relation to Distribution of Ascorbic Acid in Snap Beans: The influence of the degree of maturity on the ascorbic acid content of string beans is very different from any other seed-pod type of vegetable so far investigated. The results indicate that the variation is greatest with the larger seeded varieties (Table 3). This suggests that the effect may be due to the distribution of vitamin C between the seed and the seed pod.

To investigate this point the beans were shelled and the seeds and seed pods analyzed separately. It was found that on a fresh-weight basis the seeds contained from three to four times as much ascorbic acid as the seed pods. Hence the ascorbic acid content due to the seeds increases with growth, while that due to the shelled pods decreases

TABLE 3

Influence of Maturity on Vitamin C Content of Snap Beans

Variety	Ascorbic acid content at each stage of maturity		
	Immature	Mature	Overmature
	mg. per gm.	*mg. per gm.*	*mg. per gm.*
Tendergreen	.24	.24	.24
Ideal Market	.27	.20	.23
New McCaslin	.23	.19	.18
Refugee	.22	.19	.23
Kentucky Wonder	.25	.18	.22
Kidney Wax	.21	.18	.26
Kentucky Wonder Wax	.27	.19	.27

rapidly at first and then remains about constant. The net effect is a minimum ascorbic acid content at the usual stage of maturity at which snap beans are harvested (Table 4).

Influence of Soil Type, Fertilizer, and Other Conditions of Growth: In these experiments carefully controlled conditions of growth were required; hence, the beans were grown in a greenhouse. Two soil types, an Ontario clay loam and a muck soil, were used; both soils were previously adjusted to the same pH (6.7-6.8). The variety selected for study was U. S. No. 5, and the plants were grown in the early spring (February 20 to May 5).

The beans grown on muck soil contained .19 mg. of ascorbic acid per gram, while those grown on clay loam contained only .13 mg. of ascorbic acid per gram. These results are directly opposite to those reported previously by Tressler, Mack and King (1936) with spinach. In that case, however, the plants were grown in the field; for that reason the soil, moisture, and other conditions of growth varied widely; and the results are not strictly comparable. It is

interesting to note that, in further greenhouse experiments with spinach and radish plants grown on these same two soil types, the plants grown on muck soil were in every instance considerably higher in vitamin C than those grown on clay loam soil. It is probable that the soil type is not a primary factor influencing the vitamin C content of plants but that it affects other growing conditions which regulate directly the synthesis of vitamin C in the plant.

Duplicate plats of the clay loam soil in a greenhouse bench were treated with no fertilizer, and with 500 pounds per acre of a balanced fertilizer (4-16-4), a high nitrogen fertilizer (12-16-4), and a low nitrogen fertilizer (0-16-4). The fertilizer was finely ground and worked uniformly into the top soil. Similar plats of the muck

TABLE 4

Distribution of Vitamin C in Snap Beans

Variety	Stage of maturity	Canners U. S. standard size No.	Ascorbic acid content in			Weight in seeds
			Whole pod	Seed pod only	Seeds only	
			mg. per gm.	*mg. per gm.*	*mg. per gm.*	*pct.*
Refugee	Immature	2	.22	.20	.69	2
Refugee	Mature	4	.19	.17	.49	8
Refugee	Overmature	6	.26	.19	.43	26
New McCaslin	Overmature	5-6	.18	.18	.45	2

soil were prepared in the same way except that the amount of potash in the fertilizer was increased, the ratios being (4-16-12), (12-16-12), and (0-16-12), respectively.

The ascorbic acid contents in milligrams per gram of the beans grown on the eight different plats of clay loam soil were .15, .12, .13, .14, .12, .13, .14, .12 (average $.13 \pm .005$); while those of the beans grown on the muck soil were .19, .17, .19, .20, .19, .20, .19, .20 (average $.19 \pm .005$). Obviously these values fail to indicate any significant difference which could be attributed to the fertilizer treatment. Similar results were also obtained with spinach and radishes.

Pfützer and Pfaff (1935) found that the vitamin C content of fertilized and unfertilized plants was generally the same, but von Hahn and Görbing (1933) reported marked differences. However, a careful analysis of the data of von Hahn and Görbing shows that the differences were in most cases small and within the experimental error of the biological assay method used.

The date of maturity appears to have an effect which is superimposed upon that caused by varietal differences (Table 2). Fur-

ther experiments upon the effect of date of sampling were obtained from Refugee beans picked from the same plants on four different dates during 1937. These effects may possibly be due to differences in growing conditions, such as temperature and the relative amounts of light and moisture available.

Analyses of the Refugee variety over a period of three years show little variation in comparison to the seasonal differences obtained in this laboratory with spinach, peas, and tomatoes.

Loss of Ascorbic Acid During Storage: The storage tests show that snap beans lose their vitamin C rapidly at all temperatures investigated. The rate of loss is somewhat less at lower temperatures (Table 5).

TABLE 5

Effect of Storage on Ascorbic Acid Content of Snap Beans

Variety	Original ascorbic acid content	Ascorbic acid remaining after storage for days indicated			
		1	2	3	6
Temperature 1-3°C.(33.8-37.4°F.)		*pct.*	*pct.*	*pct.*	*pct.*
Kidney Wax	.18	66	50	44	35
Kentucky Wonder	.18	93	81	69	47
Ideal Market	.20	98	85	81	52
Refugee	.17	97	93	85	58
Temperature 8-10°C.(46.4-50°F.)					
Kidney Wax	.18	55	35	25	23
Kentucky Wonder	.18	77	62	50	33
Ideal Market	.20	82	60	49	29
Refugee	.20	89	79	68	50
Temperature 21-23°C.(69.8-73.4°F.)					
Kidney Wax	.18	40	24	20	19
Kentucky Wonder	.18	59	38	28	22
Ideal Market	.20	71	53	42	23
Refugee	.17	54	51	49	42

The Kidney Wax variety exhibited the most serious losses. The Ideal Market and Kentucky Wonder varieties kept somewhat better, especially at the lower temperatures and during the first two days of storage. The Refugee variety kept best of all, particularly at the higher temperatures.

In general it may be said that the retention of vitamin C during storage is roughly parallel to the retention of those factors which determine the quality of the vegetable. In comparison with other vegetables snap beans lose their vitamin C very rapidly in storage, especially at low temperatures. From a public-health standpoint, these results emphasize the importance of prompt marketing for this vegetable.

In our experiments the relative humidity was between 60 and 70 per cent in all the storage chambers, while in commercial cool storage it is usually maintained at practically 100 per cent. A study of the effect of humidity on losses of vitamin C during storage is being planned in this laboratory.

Loss of Ascorbic Acid During Cooking: Two varieties, Kentucky Wonder and Refugee, were selected for this experiment. The cooking procedure was that of Halliday and Noble (1933).

TABLE 6

Effect of Cooking on Ascorbic Acid Content of String Beans

Time of sampling	Original ascorbic acid remaining in		
	Drained solids	Cooking water	Total amount
	Refugee Variety [1]		
min.	*pct.*	*pct.*	*pct.*
0	100	0	100
2 (boil)	90	8	98
7	85	13	98
12	73	24	97
17 (done)	62	26	88
22	58	26	86
27	53	30	83
	Kentucky Wonder [1]		
0	100	0	100
2 (boil)	92	7	99
7	81	20	101
12	74	26	100
16	71	28	99
19 (done)	66	32	98
24	56	34	90

[1] Original ascorbic acid content of Refugee beans was .17 mg. per gm.; of Kentucky Wonder beans, .22 mg. per gm.

The results indicate that very little ascorbic acid is lost during boiling. About one-third of the original ascorbic acid is extracted into the cooking water, however. Each figure represents the mean of four separate determinations from which the average deviation was less than five per cent (Table 6).

SUMMARY

The vitamin C content of thirteen varieties of snap beans was determined. The earliness of maturity appeared to have a favorable effect upon the ascorbic acid content of the beans. This effect is superimposed upon the varietal differences.

The influence of the degree of maturity on the vitamin content of seven of the varieties was measured. It was found that the differences should be ascribed to the distribution of ascorbic acid between the shell and the seeds in the pod.

Some experiments on the influence of soil type and fertilizer treatment under controlled conditions in a greenhouse are reported.

The loss of vitamin C during storage at three different temperatures was determined.

Cooking tests were performed which showed that very little ascorbic acid is destroyed during boiling but that a considerable portion is extracted into the cooking water.

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VARIATIONS IN ASCORBIC ACID CONTENT OF BANANAS

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A considerable number of observations, both experimental and clinical, have demonstrated that the banana is a good source of vitamin C. Lewis (1919), as early as 1919, reported that 10 to 15 grams of banana pulp daily constituted a protective dose for guinea pigs against scurvy. Givens, McCluggage, and Van Horne (1921) found 10 grams of banana fully protective; and later Jansen and Donath (1925), using an Indian variety of banana, stated that 10 grams were adequate and that five grams in some cases were sufficient to prevent scurvy in experimental animals. It is now known that the two earlier workers used basal diets deficient in essential factors other than vitamin C and furthermore that they did not test dosages below 10 grams. However, other investigators—Dalldorf (1931), Gruninger (1928), and Naeslund (1931)—have contributed data which also indicated that one minimum protective dose was contained in 10 grams of ripe banana pulp. Eddy and Kellogg (1927), using the Sherman-La Mer method, found that five grams of ripe banana were equivalent to one minimum protective dose. Later, Eddy (1929) repeated this work, employing a modification of Höjer's technique, and discovered that exactly twice this amount of banana pulp, or any other antiscorbutic he used, was necessary as a minimum protective dose. More recently, Leverton (1937), using the indophenol titration technique, made a study of variations in the ascorbic acid content of bananas during ripening and found .061, .063, and .073 mg. ascorbic acid per gram of banana pulp at the green, yellow, and fully ripe stages, respectively. Harris and Poland (1937) carried out ascorbic acid titrations in parallel with a bioassay for vitamin C and reported that all of the physiologically active ascorbic acid was not detected by the usual titration procedure.

In the medical literature bananas are frequently recommended as a milk modifier and as a source of adequate vitamin C for infants and children. Johnston (1927) reported the cure of scurvy in an eight-month-old infant using banana pulp beaten into milk as the only food and antiscorbutic. The dosage represented about 250

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grams of banana pulp per day. A 19-month-old baby was cured, according to von Meysenbug (1928), of severe scurvy by adding banana pulp to its diet, as the sole source of vitamin C, in gradually increasing doses from one teaspoonful up to one whole banana (approximately 100 grams). Siperstein (1931) reported the successful use of bananas in the diets of infants and children over a period of three years. Ripe banana was incorporated in the milk formula as early as the third month and was preferred to other antiscorbutics.

Thus, although the banana is well established as a good fruit source of vitamin C, practically nothing is known concerning the normal variations in vitamin C content or the variations owing to cooking treatment, slicing with subsequent exposure to air for varying lengths of time, and dehydration. Eddy and Kellogg (1927), however, found that bananas which had been baked in their peels retained all of their original vitamin C potency but that those baked after peeling lost 67 per cent of their vitamin C.

The experiments described in this paper were undertaken to obtain data concerning the ascorbic acid variations in bananas (1) at various stages of ripeness, (2) from different tropical sources, (3) prepared for human consumption in various ways, viz., sliced, cooked, etc., and (4) in the form of commercial banana powder.

EXPERIMENTAL PROCEDURE

The extraction and titration procedure used throughout these experiments was essentially that of Bessey and King (1933) as modified by Ahmad (1935) and by Mack and Kertesz (1936). A weighed portion of the fresh fruit was thoroughly, and as quickly as feasible, macerated with 25 ml. of warm acid solution (eight per cent acetic plus two per cent metaphosphoric acid). The mixture was centrifuged and the extract decanted. Two subsequent treatments with a 10-ml. and a 5-ml. portion, respectively, of the acid solution were carried out on the residue. The extracts were combined and made up to 50 ml. with the acid reagent. This solution was then used to titrate against dilute 2, 6-dichlorophenolindophenol. The dye solution was standardized frequently against pure crystalline ascorbic acid. Parallel determinations of reducing potency of these extracts by iodine titration were also carried out but no correlation between total reducing values from iodine titrations and ascorbic acid values from the indophenol titrations developed.

A bioassay of banana pulp, after the manner of Harris and Poland (1937), showed five grams induced growth and antiscorbutic potency equal to that of .5 mg. of ascorbic acid. Daily titration of the assayed

bananas, however, indicated that an average daily intake of 7.1 grams of pulp should have been necessary to provide .5 mg. of ascorbic acid to the test animals. This suggested that (1) some of the ascorbic acid was destroyed during the time between peeling the fruit and titration, or (2) a portion of the vitamin C existed in the fruit as dehydroascorbic acid, physiologically potent but not detected by titration, or (3) some of the ascorbic acid was naturally combined with other substances which masked it during titration.

A five-minute steam treatment of the pulp, immediately upon peeling, for the purpose of inactivating any ascorbic acid oxidase, produced no change in titration values and indicated that enzymatic destruction was apparently not a factor. The use of metaphosphoric acid precluded destruction of ascorbic acid by metallic catalysis. Furthermore, the fact that occasional portions of the banana supplements remained in the guinea pig cages exposed to air for as long as six hours without apparent loss of physiological potency seemed to eliminate destruction of vitamin C as a factor in explaining the disagreement between bioassay and titration results.

To determine the amount of dehydroascorbic acid present in banana pulp a portion of the acid extract was treated with hydrogen sulfide by bubbling the gas through the solution. Subsequent titration of the reduced extract resulted in an increase in titration value of 35 to 40 per cent over an equal unreduced portion of the extract. In many other estimations of dehydroascorbic acid in bananas at several different stages of ripeness, it was found that this same percentage increase occurred. Stone (1937), using essentially the same technique, found a 34-per cent increase in reducing power after reduction with hydrogen sulfide. This indicated that approximately 71 to 74 per cent of the total vitamin C potency existed in the active ascorbic acid state while 26 to 29 per cent occurred as reversibly oxidized ascorbic acid, dehydroascorbic acid. Further credence was given to this distribution when it was remembered that banana pulp which indicated by titration an ascorbic acid value of 7.03 mg. per cent actually contained by bioassay, 10 mg. per cent or a 42-per cent increase, Harris and Poland (1937). Consequently it was assumed that a portion of the total vitamin C of bananas existed as dehydroascorbic acid; and although this amount was practically constant, that for accurate estimation of vitamin C by titration, a reduction step must be included in the procedure.

If some of the ascorbic acid occurred naturally bound or esterified—Guha and Pal (1936) and McHenry and Graham (1935)—the presence of such a compound could be detected by noting the increase in titration value resulting from heating the substance before

extraction. Bananas heated, either in the presence or absence of air, showed no tendency to increase in titratable ascorbic acid content indicating that, in this fruit at least, no ascorbic acid occurred in a heat-labile, bound form. Mack and Tressler (1937) have recently shown that the increase in titration value, when it does occur, is due to inactivation of ascorbic acid oxidase and to the decomposition of dehydroascorbic acid to a reducing substance, non-potent physiologically. These same investigators and Thornton (1938), using bananas, presented data which indicated that all of the vitamin C potency could be shown to exist as free ascorbic acid if the correct extraction medium were employed and an extremely low pH maintained. They used sulfuric acid as their principal extraction reagent.

TABLE 1

Ascorbic Acid Variations in Normally Ripened, Ripe Bananas From Different Sources

Tropical origin	Number of analyses	Range of values	Average value	International vitamin C unitage
		mg. per gm.	*mg. per gm.*	*I.U. per oz.*
Costa Rica	58	.143-.089	.109	61.8
Honduras	30	.102-.096	.100	56.7
Columbia	35	.142-.084	.110	62.4
Ecuador	25	.113-.092	.102	57.8

In the present experimental data, values should be comparable with those obtained by extraction with strong acids since reduction with hydrogen sulfide after extraction was usually carried out. In some few cases the reduction step was omitted and the final titration result was increased 37.5 per cent to represent the dehydroascorbic acid which originally was thought to be present in the fruit. It is now believed that rapid enzymatic oxidation of a portion of the naturally occurring ascorbic acid to dehydroascorbic acid during preparation and manipulation of the material for analysis was responsible for the vitamin C of the banana not detected by the titration technique, unless the reduction step was employed before titration.

DISCUSSION OF RESULTS

Bananas (*Musa sapientum*, var. Gros Michel) grown in various tropical countries contained approximately the same amount of ascorbic acid (Table 1). Regardless of their tropical source, therefore, well-ripened bananas apparently did not differ significantly in ascorbic acid potency. The value in milligrams of ascorbic acid per gram varied from .100 to .110 between the different tropical sources,

with an average of .105. This is slightly greater (five per cent) than the value found by Harris and Poland (1937) by bioassay of banana pulp from these same four tropical countries. It is considerably greater (30 per cent) than Leverton's (1937) value for ripe pulp, but considerably lower (46 per cent) than values reported by Everson and Daniels (1936) and lower than Birch, Harris, and Ray (1933) found for the banana of commerce in England. In general, from the results of this study, it may be said that ripe bananas contain ascorbic acid to give a vitamin C value of 56 to 73 International units per ounce regardless of their tropical source.

The ascorbic acid content of bananas increased during ripening with a maximum value at the stage of ripeness at which the fruit

TABLE 2

Ascorbic Acid Content of Costa Rican Bananas at Various Stages of Ripeness

Stage of ripeness	Number of analyses	Range of values	Average value	Change	International vitamin C unitage
		mg. per gm.	*mg. per gm.*	*pct.*	*I.U. per oz.*
Green peel and hard pulp	10	.058-.049	.053	.....	30.1
Green peel with some yellow	15	.066-.053	.058	9.4	32.9
Yellow more dominant in peel than green	15	.114-.038	.063	18.9	35.7
Yellow peel with green tip	20	.130-.052	.088	66.0	49.9
Completely yellow peel	20	.132-.056	.091	71.7	51.6
Yellow peel flecked with brown	18	.143-.089	.111	109.4	62.9
Black peel with overripe pulp	7	.054-.010	.032	−39.6	18.1

is usually eaten (Table 2 and Fig. 1). This increase in ascorbic acid during ripening is more pronounced than that found by Leverton (1937). In her study of bananas at three stages of ripeness, a slight trend toward increase of ascorbic acid was noted but was considered statistically insignificant. In the present study it was found that ascorbic acid in fully ripe bananas had increased 109 per cent over that present in the green fruit. A very appreciable decrease in ascorbic acid following the completely ripe stage was observed. At this stage of ripeness the peel was deep brown or black, the texture of the pulp was mushy, and the taste and aroma were somewhat disagreeable.

Since bananas are commonly served sliced and frequently some time after peeling and slicing, it was deemed advisable to determine what happened to the ascorbic acid under such conditions. Ripe bananas were thinly sliced with a nickeled spatula immediately after peeling and exposed to room temperature on china plates. Samples

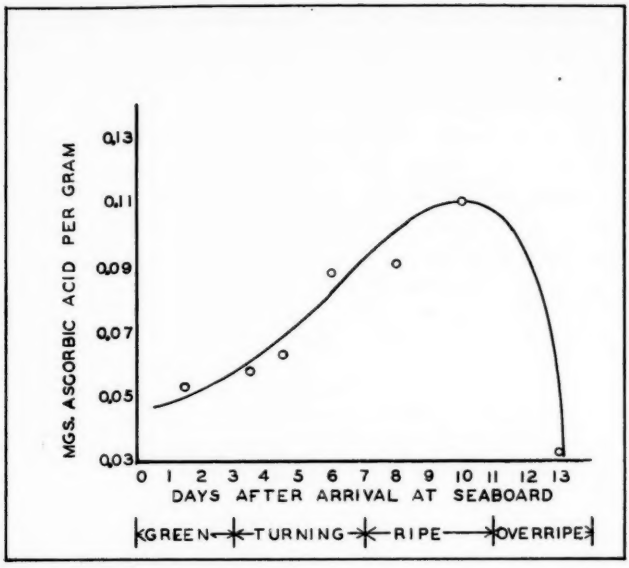

FIG. 1. Ascorbic acid content of bananas at various stages of ripeness.

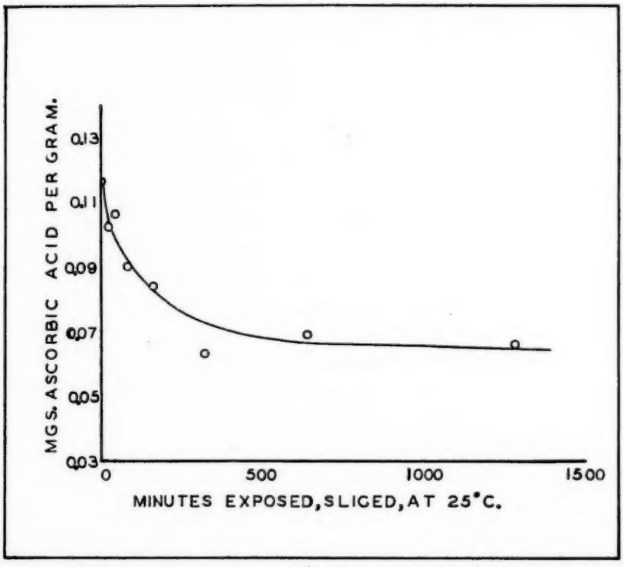

FIG. 2. Ascorbic acid content of sliced bananas exposed to room temperature for various lengths of time.

were taken at various intervals as shown (Table 3) and analyzed for ascorbic acid. The effect of this slicing treatment and subsequent exposure apparently is a loss of ascorbic acid potency which occurs almost immediately after preparing bananas by slicing (Fig. 2). After about five hours this loss becomes more or less constant at 40 to 45 per cent. In bioassays of bananas for vitamin C potency, large weighed pieces of banana pulp were fed daily to guinea pigs. In most instances this supplement was consumed immediately. However, some animals ate their banana supplement over a period of five to six hours. The fact that these animals showed no less protection from scurvy than those which ate more quickly, indicated

TABLE 3

Ascorbic Acid Content of Ripe Columbian Bananas, Sliced as for Home Use, After Different Periods of Exposure to Room Atmosphere at 25°C.(77°F.)

Length of exposure to air after slicing	Number of analyses	Range of values	Average value	Decrease	International vitamin C unitage
min.		*mg. per gm.*	*mg. per gm.*	*pct.*	*I.U. per oz.*
0	5	.142-.084	.116	.....	65.8
20	4	.129-.086	.102	—12.1	57.8
40	4	.128-.082	.106	—8.6	60.1
80	4	.113-.074	.090	—22.4	51.0
160	5	.102-.064	.084	—27.6	47.6
320	4	.080-.049	.063	—45.7	35.7
640	4	.086-.041	.069	—40.5	39.1
1,280	5	.071-.043	.066	—43.1	37.4

that exposed banana pulp underwent less loss of ascorbic acid in large pieces than when thinly sliced.

Occasionally during shipping or display, bananas are accidentally exposed to a temperature below 7.1°C.(45°F.) for 12 hours or longer. Such exposure causes an injury as evidenced by discoloration of the peel. Apparently this phenomenon is merely a peel injury since the flavor, composition, and texture of the pulp is in no way affected. However, it seemed of interest to see if the ascorbic acid content would be affected by such chilling treatment. The data (Table 4) indicate that for all practical purposes slightly chilled bananas suffered no reduction in their ascorbic acid potency. When fully ripe, the only visual difference between slightly chilled and unexposed bananas is the somewhat duller yellow color of the peel of the former. However, severely chilled bananas, as indicated by a black peel and general unsaleable condition, were seriously affected as regards ascorbic acid content. More than 50 per cent of the ascorbic

acid content was destroyed by the low temperature at each of the three stages of ripeness tested.

That ripe bananas, commercially dehydrated, suffered a considerable loss of ascorbic acid potency is also shown (Table 4). Assuming that the fruit used to manufacture the banana powders tested, contained .100 mg. ascorbic acid per gram of pulp and 75 per cent moisture, the actual loss of ascorbic acid potency would be 87 per cent for spray-dried powder and 84 per cent for drum-dried product.

TABLE 4

Ascorbic Acid Content of Ripe Hondurasian Bananas, Exposed to Low Temperatures at Various Stages of Ripeness, and of Commercially Dehydrated Banana Powder

Treatment	Stage of ripeness	Number of analyses	Average value	Change	International vitamin C unitage
			mg. per gm.	*pct.*	*I.U. per oz.*
Control	Green	2	.062	.....	35.2
Slightly chilled[1]	Green	3	.064	3.2	36.3
Control	Turning	2	.092	.....	52.2
Slightly chilled[1]	Turning	3	.089	—3.3	50.5
Control	Ripe	2	.109	.....	61.8
Slightly chilled[1]	Ripe	3	.106	—2.8	60.1
Control	Green	2	.052	.....	29.5
Severely chilled[1]	Green	3	.023	—55.8	13.0
Control	Turning	2	.088	.....	49.9
Severely chilled[1]	Turning	3	.039	—55.7	22.1
Control	Ripe	2	.110	.....	57.3
Severely chilled[1]	Ripe	3	.051	—53.6	28.9
Spray-dried banana powder	Ripe	3	.051	.....	28.9
Drum-dried banana powder	Ripe	3	.063	.....	35.7

[1] Bananas which were slightly chilled had been exposed to a temperature of 7.1°C. (45°F.) for 12 to 18 hours, while the severely chilled fruit had been exposed at 1.6°C. (35°F.) for 24 hours.

This difference is not sufficiently great to permit claim of superiority for either dehydration procedure as regards effect on ascorbic acid content.

The effect of various cooking procedures upon the ascorbic acid content of both partially and completely ripe bananas is shown (Table 5). In general, it must be concluded that cooking caused an appreciable loss of ascorbic acid potency and that the degree of this loss was related primarily to the time of cooking rather than to the temperature used. The sautéed fruit was cooked in a hydrogenated vegetable oil which possessed no reducing power. The one exception

TABLE 5

Ascorbic Acid Content of Costa Rican Bananas Cooked in Various Ways

Cooking treatment	Stage of ripeness	Number of analyses	Range of values	Average value	Change	International vitamin C unitage
			mg. per gm.	*mg. per gm.*	*pct.*	*I.U. per oz.*
Control—no treatment	Yellow peel with green tip	3	.086-.102	.093	.....	52.7
Baked without skins (375°F. for 23 min.)	Yellow peel with green tip	3	.037-.067	.048	—48.4	27.2
Baked in skins (375°F. for 27 min.)	Yellow peel with green tip	3	.079-.107	.096	3.2	54.4
Broiled (550°F. for 9 min.)	Yellow peel with green tip	3	.084-.087	.085	—8.6	48.2
Sautéed (6 min.)	Yellow peel with green tip	3	.062-.074	.068	—26.9	38.6
Control—no treatment	Yellow peel with brown flecks	3	.102-.110	.107	.....	60.7
Baked without skins (375°F. for 19 min.)	Yellow peel with brown flecks	3	.042-.057	.049	—54.2	27.8
Baked in skins (375°F. for 21 min.)	Yellow peel with brown flecks	3	.060-.070	.066	—38.3	37.4
Broiled (550°F. for 9 min.)	Yellow peel with brown flecks	3	.042-.096	.073	—31.8	41.4
Sautéed (5 min.)	Yellow peel with brown flecks	3	.040-.067	.055	—48.6	31.2

to the general conclusion that ascorbic acid is destroyed by cooking was that of the partially ripe banana baked in the peel. Bananas cooked in this manner apparently retained all of their ascorbic acid. This corroborated the finding of Eddy and Kellogg (1927). It seems probable that the fully ripe bananas baked in the peel would also have retained their ascorbic acid potency had not the peel, which is very thin at this stage of ripeness, been split, owing to the cooking procedure.

We appreciate the coöperation of Miss I. S. Lindman, Home Economics Department of the Fruit Dispatch Company of New York, who cooked the fruit tested in these experiments.

CONCLUSIONS

The average ascorbic acid content of ripe bananas, regardless of their tropical source, was relatively constant at .100 to .110 mg. per gram.

Green bananas contained approximately .053 mg. ascorbic acid per gram which increased, as the fruit ripened, to a maximum (.110 mg. per gm.) at the stage of ripeness at which the banana is usually eaten, peel yellow and flecked with brown, and then rapidly decreased to .032 in the overripe state.

Bananas prepared for human consumption by cooking in various ways, suffered an inconstant, yet appreciable, loss of ascorbic acid content except when cooked in the unbroken peel, under which condition there was practically complete retention of ascorbic acid. Sliced bananas exposed to room temperature also lose some ascorbic acid potency. In about five hours the loss reached about 40 to 45 per cent and remained constant for the remainder of the 22-hour test. Banana powder, prepared commercially by either the spray process or the drum-dry process, has been shown to contain approximately 80 to 90 per cent less ascorbic acid than bananas of the same ripeness as those from which the powders were manufactured.

Slight chilling of bananas, wherein the peel was only slightly dulled in color, had no deleterious effect on the ascorbic acid content. However, severely chilled fruit, the peel becoming black and the pulp soft upon ripening, suffered a loss in ascorbic acid of over 50 per cent.

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INFLUENCE OF BACTERIA ON OXIDATION OF ASCORBIC ACID[1]

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Although the action of intestinal bacteria on ascorbic acid has been studied by a number of investigators, very little information has been reported concerning the action of bacteria commonly associated with foods on the oxidation of ascorbic acid. The purpose of this investigation was to study the effect of a miscellaneous group of bacteria, many of which may occur in foods, on the oxidation of ascorbic acid. Such a study should provide information as to whether or not bacteria may be of importance in so far as the stability of ascorbic acid in foods is concerned.

REVIEW OF LITERATURE

Stepp and Schroder (1935), Stepp (1936), Gagyi (1936), Gagyi and Ujsaghy (1936), and Kendall and Chinn (1938) reported that certain strains of intestinal bacteria were capable of fermenting or otherwise destroying ascorbic acid. Kligler, Leibowitz, and Berman (1937), and Esselen (1938a, 1938b) reported that certain bacteria were capable of retarding the rate of oxidation of ascorbic acid. After an intensive study of intestinal types of bacteria, Esselen (1938b) suggested that growing bacteria may retard the rate of oxidation of ascorbic acid by a combination of four factors: (1) the formation of un-ionized copper complexes whereby the catalytic action of copper is destroyed, (2) the production of carbon dioxide with the subsequent saturation of the medium with it, (3) the lowering of the oxygen tension of the medium, and (4) the lowering of the oxidation-reduction potential of the medium.

Some work has been reported relative to the effect of microbial growth on the ascorbic acid content of foods. Lepkovsky, Hart, Hastings, and Frazier (1925) found that *Streptococcus lactis* had no effect on the vitamin C content of orange and tomato juices. Bifano and Servazzi (1935) "infected" lemons with *Penicillium digitatum* and found that their vitamin C content was the same as that of non-infected lemons.

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It would thus appear that the presence of bacteria in foods should have no harmful effect on the ascorbic acid present unless they are capable of actually fermenting it.

EXPERIMENTAL PROCEDURE

Throughout this investigation nutrient broth and nutrient broth plus .1 per cent of glucose were used. The composition of the nutrient broth was as follows:

"Bacto" beef extract	3 grams
"Bacto" peptone	5 grams
Glucose (when used)	10 grams
Distilled water	1,000 c.c.
Reaction	pH 6.3 - 6.5.

The media were prepared and distributed in 100-ml. quantities in 250-ml. Erlenmeyer flasks and sterilized. A 24-hour, nutrient-broth culture of the organism being studied was used for inoculation, one milliliter of the liquid culture being added to each flask. An incubation temperature of 37°C.(98.6°F.) was used. Pure crystalline ascorbic acid was employed, and it was added to the medium in aqueous solution which was made up to contain the desired amount of the acid and sterilized by filtration through a Seitz filter.

The ascorbic acid content of the medium was determined at the beginning and at the end of each experiment by a modification of the 2, 6-dichlorophenolindophenol dye-titration method of Tillmans as described by Bessey and King (1933). The actual method employed throughout this study consisted of placing 20 ml. of the culture mixture into a 50-ml. flask and adding five milliliters of glacial acetic acid (sufficient to lower the pH value of the titration mixture to 2). The acidified solution was titrated immediately with a standard solution of the 2, 6-dichlorophenolindophenol dye. The titration was completed within two minutes in all cases. For convenience all data are reported in terms of milligrams of ascorbic acid per 20 ml. of the medium, and as the percentage of loss of ascorbic acid during the incubation period.

Forty-one different bacterial strains representing 35 species were studied relative to their influence on the oxidation of ascorbic acid during a six-hour incubation period. Ascorbic acid was added to freshly inoculated and to 24-hour cultures of the organisms in both nutrient and glucose broth according to the method described above. The ascorbic acid content of the media was determined before and after a six-hour incubation period at 37°C.

TABLE 1

Influence of Freshly Inoculated and 18-Hour Cultures of Bacteria on Oxidation of Ascorbic Acid in Nutrient Broth and Glucose Broth in Six Hours at 37°C.(98.6°F.)

Organism	Nutrient broth				Glucose broth	
	Freshly inoculated culture		18-hour culture		18-hour culture	
	Ascorbic acid per 20 ml. of broth	Loss in 6 hours	Ascorbic acid per 20 ml. of broth	Loss in 6 hours	Ascorbic acid per 20 ml. of broth	Loss in 6 hours
	mgm.	*pct.*	*mgm.*	*pct.*	*mgm.*	*pct.*
Control (start)	.94	....	.92	....	1.20	....
Control (after 6 hours)	.11	88	.10	89	0.10	92
Micrococcus agilis	.09	90	.15	84	0.14	88
Micrococcus cereus	.10	89	.10	89	0.10	92
Micrococcus flavus	.36	62	.88	4	1.05	12
Micrococcus varians	.09	90	.75	19	0.12	90
Gaffkya tetragena (a)	.09	90	.06	93	0.16	87
Gaffkya tetragena (b)	.10	89	.45	51	0.62	48
Streptococcus fecalis	.12	87	.52	43	1.20	0
Staphylococcus citreus	.11	88	.10	89	0.10	92
Staphylococcus albus	.11	88	.92	0	0.93	23
Staphylococcus aureus	.11	88	.83	10	0.86	28
Sarcina aurantiaca (a)	.09	90	.60	25	0.37	69
Sarcina aurantiaca (b)	.11	88	.92	0	0.40	66
Planosarcina	.08	91	.05	95	0.10	92
Pseudomonas fluorescens (a)	.12	87	.92	0	0.95	21
Pseudomonas fluorescens (b)	.59	37	.92	0	0.95	21
Pseudomonas syncyanea (a)	.28	70	.84	9	1.20	0
Pseudomonas syncyanea (b)	.32	66	.19	80	0.99	17
Serratia marcescens	.45	52	.92	0	1.20	0
Chromobacterium violaceum (a)	.10	89	.84	9	0.85	29
Chromobacterium violaceum (b) from fish	.15	84	.80	13	1.15	4
Achromobacter viscosum	.09	90	.83	10	0.98	18
Aerobacter (encapsulated, from oysters)	.31	67	.92	0	1.12	7
Proteus vulgaris	.11	88	.89	3	0.83	31
Alcaligenes fecalis	.08	91	.06	93	0.08	93
Spirillum rubrum	.11	88	.72	22	0.80	33
Bacillus subtilis	.09	90	.57	38	1.20	0
Bacillus cereus	.13	86	.68	26	0.75	37
Bacillus mycoides	.14	85	.79	14	0.30	75
Bacillus megatherium (a)	.10	89	.72	22	1.15	4
Bacillus megatherium (b)	.11	88	.68	26	0.90	25
Bacillus albolactis	.16	83	.82	11	0.95	21
Bacillus simplex	.31	67	.92	0	1.07	11
Bacillus prausnitzii	.11	88	.53	42	0.40	66
Bacillus (from ropy milk)	.45	52	.92	0	1.20	0
Torula rosea	.09	90	.17	82	0.53	56
Aerobacter aerogenes	.54	49	....	....	1.18	2
Escherichia coli	.67	36	.92	0	1.20	0
Escherichia neapolitana	.82	19	....	....	1.20	0
Escherichia acidi-lactici	.47	57	....	....	1.20	0
Aerobacter cloacae	.38	68	....	....	1.20	0
Streptococcus lactis	.18	88	....	....	0.38	69

DISCUSSION OF RESULTS

As may be seen from the experimental data (Table 1) most of the organisms exerted a pronounced inhibitory action on the oxidation of ascorbic acid. It was evident from the appearance of the cultures that those organisms which grew most rapidly exerted the strongest inhibitory action on the oxidation of ascorbic acid. The presence of a readily fermentable carbohydrate, such as glucose, in the medium still further enhanced the ability of the bacteria to retard the oxidation of ascorbic acid. As has been previously observed by Esselen (1938b), 24-hour cultures of bacteria exerted a more marked inhibitory action on the oxidation of ascorbic acid than did freshly inoculated cultures of the same organisms. Ascorbic

TABLE 2

Influence of Anaerobes and of Lactobacillus acidophilus on Oxidation of Ascorbic Acid

Organism	Ascorbic acid per 20 ml. of broth	Loss of ascorbic acid in 6 hours at 37°C. (98.6°F.)
	mgm.	*pct.*
Control (start)	.66	....
Control (after 6 hours)	.10	85
Clostridium welchii	.58	12
Clostridium sporogenes	.57	14
Clostridium botulinum	63	5
Control (start)	.93	....
Control (after 6 hours)	.75	19
Lactobacillus acidophilus	.85	9

acid determinations on control cultures of the bacteria studied, with no ascorbic acid added, gave no indication that ascorbic acid or ascorbic acid-like substances were synthesized by the bacteria.

In addition to the above bacteria, the influence of three species of *Clostridia* and of *Lactobacillus acidophilus* on the oxidation of ascorbic acid were investigated. *Clostridium welchii, Clostridium sporogenes,* and *Clostridium botulinum* were grown in glucose broth under anaerobic conditions. Their ability to inhibit the oxidation of ascorbic acid during a six-hour incubation period at 37°C. was determined (Table 2). The results obtained with *Lactobacillus acidophilus* are also presented in this table. This organism was grown in skim milk in an atmosphere of ten per cent of carbon dioxide. The ascorbic acid determinations on the milk cultures were made by the method of Sharp (1938). In this method an aliquot portion of the culture is acidified with .1 N sulphuric acid and the mixture is

titrated immediately with the 2, 6-dichlorophenolindophenol dye solution. As was found by Sharp, the presence of the coagulated milk protein in the titration mixture did not interfere with the titration. Because of its slow growth, 48-hour cultures of *Lactobacillus acidophilus* were employed.

It may be noted that *Clostridium welchii, Clostridium sporogenes, Clostridium botulinum,* and *Lactobacillus acidophilus* all exerted an inhibitory action on the oxidation of ascorbic acid (Table 2). Skim milk itself was found to exert a considerable inhibitory action on the oxidation of ascorbic acid as compared with the other culture media used in this investigation.

SUMMARY

Forty-five different strains of bacteria, representing 39 species, were studied with reference to their ability to retard the oxidation of ascorbic acid. It was found that most of these organisms significantly retarded the oxidation of ascorbic acid, especially when they were grown in a medium containing a readily fermentable carbohydrate. No evidence was obtained which would indicate that bacteria are destructive to ascorbic acid. Thus it would appear that bacteria are not of importance in causing a loss of ascorbic acid in foods.

Appreciation is due Dr. James E. Fuller of the Department of Bacteriology for his interest in this investigation.

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A COMPARISON OF GELATIN AND SODIUM ALGINATE[1] AS STABILIZERS IN ICE CREAM

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The use of a stabilizing agent in the manufacture of ice cream has been a common practice for some time. The *Ice Cream Trade Journal* in its first issue carried advertising and contained an article by Alberts (1905) explaining the merits of gelatin as a constituent of ice cream. Washburn (1910) stated that gelatin had been used in commercial ice cream for a great many years. Today practically all commercial ice cream contains a stabilizing agent of some kind.

Stabilizers function in ice cream through certain of their colloidal properties which affect the size and nature of the ice-crystal formation during the freezing and hardening process. To be satisfactory for use, the stabilizer should be tasteless, odorless, easily dissolved in the mix, free from impurities, impart desirable melting characteristics to the ice cream, and should not seriously affect the whipping time of the mix. The nutritive properties and cost of the stabilizer naturally are also of some importance.

Gelatin, a by-product of the packing industry, is a protein derived mostly from the collagen contained in beef and pork skins and body tissues. It is a typical colloid of the emulsoid type, a characteristic that enables it to exert a protective action upon crystals formed in its presence, causing them to come down in a finely divided and suspended form. According to Dahlberg (1925), however, "The real value of gelatin in ice cream is due to the formation of a gel during the freezing process or immediately thereafter in the hardening room, which, on account of its stiffness and internal structure, prevents the growth of large ice crystals that cause coarse ice cream."

The popularity of gelatin in ice cream has naturally encouraged the introduction of competitive products, mostly of vegetable origin. Various types of gums and starches have been tried with a limited degree of success. A few years ago a product called "Kelco-Gel," a derivative of a sea weed, was studied in the laboratories of the University of Illinois, but was found unsatisfactory because of its effect upon flavor and color of ice cream. Later, this product, in a

[1] The trade name for this product is Dariloid. The material was identified as sodium alginate using tests outlined in Allen Commercial Organic Analysis, Vol. 10, pp. 187-189.

more refined form, was sold as Dariloid for use in ice cream, and Cocoloid for use as a stabilizer in chocolate milk. Following the introduction of this product, Goodman (1935) reported on the use of sodium alginate in ice cream. He stated that only about 60 per cent as much sodium alginate is necessary in proportion to amounts required of other stabilizers—.15 to .25 per cent giving satisfactory stabilization of the ice cream. Goodman further claimed sodium alginate capable of producing better whipping of the mix, which he explained as being due to the reduced amount of calcium caseinate formed in the mix resulting from a combination of the calcium caseinate with the sodium alginate, producing sodium caseinate and calcium alginate.

Sommer (1935a) makes the statement that .25 per cent Dariloid was equal or slightly superior to .40 per cent 225 Bloom gelatin, and that .20 per cent Dariloid was equal or slightly superior to .30 per cent 225 Bloom gelatin. Later, Mack (1936) reported that sodium alginate was more effective than gelatin of average strength in improving texture and produced a faster whipping mix. Stebnitz and Sommer (1937) reported that under ideal freezing conditions and when the gelatin content was low there was little choice between the two stabilizers in regard to whipping, but under the reverse conditions the sodium-alginate mixes showed the better whipping ability. Anderson, Dowd, and Helmboldt (1937) found that sodium alginate enhanced the whipping ability of the mix and produced an ice cream with a smoother, creamier melt down. The most satisfactory results were secured when using .3 per cent sodium alginate.

For the purpose of studying the claims advanced for the use of sodium alginate an investigation was begun in June, 1935. The sodium alginate used was supplied by the Kelco Company, Chicago, Illinois. The gelatin used was furnished in part by the Edible Gelatin Association; a part of the remainder was purchased from a company manufacturing from pig-skin stock and the other was secured from a calf-skin stock factory.

EXPERIMENTAL PROCEDURE

In the first experiments the batches were of 60- or 100-pound size and were made from 40 per cent cream, sweetened condensed skim milk containing 28 per cent serum solids and 42 per cent sugar, skim milk, sugar, and the necessary amount of stabilizer. The cream, condensed milk, and skim milk necessary for all the mixes in each run were mixed and heated to 62.8°C.(145°F.) for 30 minutes and homogenized at that temperature with a pressure of 2,500 pounds on the first valve and 500 pounds on the second valve. The homog-

enized mixture of cream, condensed milk, and skim milk was immediately returned to the pasteurizer without cooling. Enough of this basic mix was then weighed out to make 60- or 100-pound batches, after adding the necessary stabilizer, sugar, and water. For the gelatin mixes, the mixture of basic mix, sugar, and gelatin was heated to 71.1°C.(160°F.) for six minutes in 10-gallon milk cans placed in a hot-water bath, the mixture being stirred constantly. In the case of the sodium-alginate mixes, the basic mix was heated to 160°F. before the necessary amounts of sugar and sodium alginate were added. It was then mixed thoroughly for six minutes, taking care not to permit the temperature to drop below 160°F., so as to facilitate the dissolving of the sodium alginate. In all cases the stabilizer was mixed with a part of the sugar used in the mix and added by sifting it dry into the basic mix. The mixes were cooled to below 10°C.(50°F.) by pouring over a surface cooler. Both 12- and 16-per cent fat mixes were used in the study.

A part of each batch was frozen within four hours after processing, and the other portion was frozen after aging at 4.4°C.(40°F.) for 20 to 24 hours. A 40-quart, direct-expansion freezer was used. An attempt was made to freeze all the experimental batches to the same minimum temperature in the freezer. Overrun and temperature readings were taken each minute during the freezing and whipping process. Samples were taken at 100 per cent overrun for judging and melting tests.

Viscosity measurements were made of the mixes before and after aging at approximately 40°F., using a MacMichael viscosimeter, with a No. 26 wire, the bowl traveling at a speed of 50 R.P.M., and measurements are expressed in degrees MacMichael. In each case the mix was treated to remove the apparent viscosity by pouring from one container to another before making the viscosity measurements.

Melting tests were run in duplicate at room temperature. The samples were weighed each 30 minutes to determine the relative amounts melted.

Color changes occurring in the mix as a result of use of sodium alginate were measured with a K. and E. color analyzer, manufactured by the Keuffel and Esser Company. The range of wave lengths used was 480 to 630 inclusive, with 30-millimicron intervals.

PRESENTATION OF DATA

The accumulated data are too voluminous to present in entirety. Comparisons were made between the two types of stabilizers when used in varying quantities of each. Both 175 and 225 Bloom gelatin was used, in both 12- and 16-per cent fat mixes.

Under the conditions of the experiments, sodium alginate and gelatin gave somewhat different results in ice cream. In the first place, while gelatin can be added to the mix before pasteurization and can be completely incorporated at ordinary pasteurizing temperature (145°F.), sodium alginate is best dispersed in the hot mix at a temperature of at least 160°F. The viscosity measurements indicate that the action of the sodium alginate is immediate, whereas the gel formation of the gelatin is known to be a function of time and the temperature of the solution. The sodium alginate mixes reached a peak viscosity within a few hours after cooling, while the gelatin mixes required approximately two days for this to occur.

That ice cream containing sodium alginate melts down more rapidly when exposed to room temperature than does ice cream containing gelatin is very evident from the data.

Both stabilizers increase the time required to reach 100 per cent overrun, in both the unaged and aged mixes, as compared with the control mixes. In no case did either stabilizer improve the whipping qualities of the mix. The whipping qualities of the mixes containing gelatin and sodium alginate varied so slightly that it cannot be said that one stabilizer was distinctly more detrimental to whipping than the other, when used in the proportions necessary to give comparable body scores.

In this connection a summary of the freezing data and the fresh ice cream body scores is of interest. This summary (Table 1) shows that in the case of the unaged mixes two of the six sodium-alginate mixes whipped to 100 per cent overrun sooner than the gelatin mixes, while the average time required to reach 100 per cent overrun at the draw was .21 minute faster in the case of the gelatin mixes. After aging there was only one case where the sodium-alginate mixes whipped faster, while the average time to reach 100 per cent overrun was .06 minute less in the case of the gelatin mixes. Such differences are too small to justify attributing superior whipping qualities to either stabilizer. To be true, if smaller amounts of either stabilizer had been used the resulting whipping qualities of that mix probably would have shown up more favorably. It is important, therefore, to compare the body scores of the ice creams in relation to the whipping periods. When comparing data on the unaged mixes it will be noted that the average body scores are practically the same. In three instances the gelatin ice creams scored higher, while in three other cases the sodium-alginate ice creams scored higher. In the case of the aged mixes, however, the gelatin ice creams were placed first in every instance, averaging four-tenths of a point higher in score than did the sodium-alginate ice creams.

The data show that aging improved the whipping of all mixes and raised the body scores of all ice creams (Table 1), although there was less change in the body scores of the ice creams stabilized with sodium alginate as a result of the aging than in the case of the ice cream stabilized with gelatin.

The data giving the comparative body scores of ice creams stabilized with sodium alginate and 175 to 225 Bloom gelatins (Table 2) show that when the mixes were unaged the average scores for the

TABLE 1

Summary of Freezing Data and Body Scores

	Gelatin mixes [1]			Sodium-alginate mixes		
Fat mix	Amount	Time to reach 100% overrun	Body score of fresh ice cream	Amount	Time to reach 100% overrun	Body score of fresh ice cream
	pct.	*min.*		*pct.*	*min.*	
1. 12% fat	.35	9.25	23.50	.30	9.50	23.00
2. 12% fat	.35	8.25	22.75	.30	8.00	23.00
3. 12% fat	.35	6.75	23.00	.30	6.50	22.75
4. 16% fat	.30	7.50	23.50	.25	9.00	23.75
5. 16% fat	.30	8.20	23.85	.30	8.75	23.75
6. 12% fat	.35	6.75	23.25	.30	7.25	23.15
Average	.....	7.55	23.31	.....	8.16	23.23
Mixes aged						
1. 12% fat	.35	6.50	23.50	.30	6.50	23.00
2. 12% fat	.35	7.25	23.50	.30	7.00	23.00
3. 12% fat	.35	6.50	23.25	.30	6.75	23.00
4. 16% fat	.30	7.25	24.25	.25	7.50	23.75
5. 16% fat	.30	8.20	24.25	.30	8.25	24.10
6. 12% fat	.35	6.50	23.75	.30	7.00	23.25
Average	.....	7.17	23.75	.....	7.23	23.35

[1] Includes 215-225 Bloom gelatins.

ice creams containing sodium alginate and those containing the 225 Bloom gelatin were the same, while in the case of the aged mixes the average was somewhat higher for the gelatin ice creams. The ice creams containing the lower-test gelatin, however, had a higher body score than did the sodium-alginate ice creams made from both the aged and unaged mixes. This slightly better showing of the lower-test gelatin might have been due to the fact that this gelatin was added somewhat in excess (.04% in the 12% fat mixes and .035% in the 16% mixes) of what it should have been to cost the same as the amount of 225 Bloom gelatin used.

TABLE 2

Comparison of Body Scores of Ice Cream Stabilized With Sodium Alginate, 175 and 225 Bloom Gelatins, Stored Under Different Conditions

Part 1—12% fat mixes

Ice cream	Unaged mixes					
	.3% sodium alginate		.35% 225 Bloom gelatin		.45% 175 Bloom gelatin	
	Body scores Batch a	Batch b	Body scores Batch a	Batch b	Body scores Batch a	Batch b
1-2 days after freezing	23.00	22.75	22.75	23.00	23.00	23.25
Stored at —23.3 to —17.8°C. (—10 to 0°F.) 7 days	23.00	22.75	22.75	23.00	23.00	23.25
Stored at —17.8 to —12.2°C. (0 to 10°F.) 7 days	22.50	22.00	22.00	22.50	22.25	22.75
Average	22.67		22.67		22.92	
Aged mixes						
1-2 days after freezing	23.00	23.00	23.50	23.25	23.25	23.50
Stored at —23.3 to —17.8°C. (—10 to 0°F.) 7 days	23.00	23.25	23.50	23.00	23.25	23.50
Stored at —17.8 to —12.2°C. (0 to 10°F.) 7 days	22.75	22.85	23.50	23.00	23.00	23.50
Average	22.98		23.31		23.33	

Part 2—16% fat mixes

Ice cream	Unaged mixes					
	Pct. sodium alginate		.3% 225 Bloom gelatin		.385% 175 Bloom gelatin	
	.25	.30				
1 day after freezing	23.75	23.75	23.50	23.85	23.75	24.00
Stored at —23.3 to —17.8°C. (—10 to 0°F.) 15 days	23.60	23.75	23.50	23.85	23.75	24.00
Stored at —17.8 to —12.2°C. (0 to 10°F.) 15 days	23.50	23.75	23.25	23.85	23.50	24.00
Average	23.68		23.63		23.83	
Aged mixes						
1 day after freezing	23.75	24.10	24.25	24.25	24.00	24.00
Stored at —23.3 to —17.8°C. (—10 to 0°F.) 15 days	23.75	24.00	24.25	24.25	24.00	24.15
Stored at —17.8 to —12.2°C. (0 to 10°F.) 15 days	23.50	23.75	24.00	24.00	23.75	23.90
Average	23.83		24.17		23.97	
Grand average	23.29		23.45		23.51	

It is also of interest to note (Table 2) that the ice creams containing sodium alginate withstood storage-temperature fluctuations with practically the same degree of body deterioration as did those containing gelatin. This is shown by the following averages of the body scores of the ice creams stored under different conditions as compared with the original scores.

Stabilizer used	Average score fresh	Average loss in score after storage	
		—23.3 to —17.8°C. (—10 to 0°F.)	—17.8 to —12.2°C. (0 to 10°F.)
Sodium alginate	23.39	.00	.31
225 Bloom gelatin	23.59	.08	.23
175 Bloom gelatin	23.60	.01	.27

It was observed that the sodium alginate resulted in an ice cream with a drier body than when gelatin was used. Especially was this true when an excess of the alginate had been added.

EFFECT OF INCREASED AMOUNTS OF SODIUM ALGINATE

While the data averages given show a very slight advantage in favor of gelatin under the conditions of the experiment, this is of no particular significance since variations in the amount of stabilizer used or in the strength of the stabilizer may change this relationship entirely. For example, samples of sodium alginate studied in 1937 and 1938 indicated a somewhat greater stabilizing action than those secured in 1935. One sample secured recently from an English manufacturer seems to have a relatively higher stabilizing power than that produced in this country, as only .075 to .10 per cent is recommended for mix, or approximately one-third of the amount of sodium alginate used in these experiments. Since sodium alginate reacts with the calcium salts in the mix as well as the calcium combined with the casein to form a jelly-like substance, variation in the amount of calcium available may also affect the extent of the stabilizing action. The important thing in a study of this nature, therefore, is not a determination of how much of one will be needed to equal the other as a mix stabilizer but a consideration of the relative merits of the two when used in amounts necessary to produce proper stabilization of the mix.

In experiments with 12-per cent fat mixes in which the sodium alginate was varied from .3 to .4 per cent at .05-per cent intervals whereas the gelatin remained constant at .35 per cent, it was found that as the amount of the sodium alginate was increased an improved body resulted which was distinctly superior to that of the

gelatin ice cream when .35 and .4 per cent of the sodium alginate was used. In other words by proper adjustment of the amount of stabilizer used comparable body results can be obtained when using gelatin and sodium-alginate stabilizers.

It was interesting to note in these experiments that as the amount of sodium alginate was increased the viscosity of the mix increased and the rapidity of melt down at room temperature was decreased. At the higher concentration of sodium alginate there was some wheying off, indicating a destabilization of the mix casein.

RELATIVE MERITS OF SODIUM ALGINATE AND GELATIN WHEN ADDED BEFORE PROCESSING

To study the relative stabilizing properties of sodium alginate and gelatin when added to the mix before processing, an experiment was planned in which mixes containing 12 per cent fat, 10.5 per cent serum solids, 14 per cent sugar, and .3 per cent sodium alginate were compared with mixes identical except that .35 per cent gelatin (210 Bloom) was used as the stabilizer. The stabilizers were processed with the other mix constituents, the sodium alginate being added with a part of the sugar when the mix reached 71.1°C. (160°F.), while the gelatin was added mixed with a part of the sugar when the temperature of the mix reached 32.2°C.(90°F.). The sodium alginate was of a different stock than that used in the previous experiments and, as indicated by comparative viscosity effects, had somewhat more stabilizing powers.

After aging 24 hours, seven batches of each mix were frozen in a 50-quart batch freezer and 24 hours later seven batches of each mix were frozen in a 10-quart counter freezer.

Here again the gelatin mixes whipped slightly faster than the sodium-alginate mixes when frozen in the large freezer (Table 3). The maximum overrun obtainable was practically the same in both cases. When frozen on the smaller and slower counter freezer, however, somewhat more favorable results were obtained with the sodium-alginate mixes, so far as whipping time was concerned. The same trend of faster whipping of the sodium-alginate mixes when using the counter freezer was noted on two other runs. It is possible, therefore, that the superior whipping of sodium-alginate mixes, as reported by some investigators, might be explained through the type of freezer used.

Since calcium salts, when present in the mix, have been shown by Sommer (1935b) to prolong the whipping period, it is possible that such mixes when stabilized with sodium alginate, which reacts readily

TABLE 3

Comparison of .30 Per Cent Dariloid and .35 Per Cent Gelatin When Stabilizers Were Added Before Processing

Part 1—50-quart batch freezer

Sodium-alginate mixes			Gelatin mixes		
Time	Temp.	Overrun	Time	Temp.	Overrun
Mix 1					
min.	*°F.*	*pct.*	*min.*	*°F.*	*pct.*
0	52.6	0	0	47.5	0
1	34.8	21	1	30.4	32
2	26.9	33	2	26.6	38
3	26.0	45	3	25.5	50
4	24.9	60	4	24.6	58
5	24.3	67	5	24.1	68
6	24.4	90	6	24.2	100
6.5	24.45	100	....	.....	.....
Mix 2					
0	52.6	0	0	47.5	0
1	30.1	27	1	28.4	32
2	26.4	33	2	26.7	39
3	25.6	50	3	25.6	53
4	24.8	65	4	24.7	62
5	24.3	77	5	24.2	79
6	24.4	97	6	24.3	100
6.5	24.5	102	....	.....	.....
Mix 3					
0	52.6	0	0	47.5	0
1	29.8	23	1	27.8	31
2	26.1	44	2	26.5	40
3	25.5	55	3	25.4	51
4	24.8	63	4	24.5	62
5	24.3	80	5	24.1	78
6	24.4	90	6	24.2	102
6.5	24.5	100	....	.....	.....
Mix 4					
0	52.6	0	0	47.5	0
1	29.5	33	1	29.0	33
2	26.3	42	2	26.4	43
3	25.6	58	3	25.3	50
4	24.4	67	4	24.2	63
5	24.1	73	5	24.0	75
6	24.1	95	6	24.1	100
6.5	24.2	100	....	.....	.....
Mix 5					
0	52.6	0	0	47.5	0
1	30.0	31	1	28.5	31
2	26.6	43	2	26.8	38
3	25.7	58	3	25.1	50
4	24.9	67	4	24.6	61
5	24.4	78	5	24.5	79
6	24.45	96	6	24.6	101
6.3	24.5	102	....	.....	.....
Mix 6					
0	52.6	0	0	47.5	0
1	30.0	34	1	29.8	32
2	27.0	50	2	26.9	38
3	26.0	57	3	25.9	48
4	24.9	69	4	25.0	60
5	24.6	79	5	24.6	76
6	24.6	96	6	24.7	100
6.3	24.7	104	....	.....	.....

TABLE 3 *(Concluded)*

Comparison of .30 Per Cent Dariloid and .35 Per Cent Gelatin When Stabilizers Were Added Before Processing

Part 1—50-quart batch freezer

Sodium-alginate mixes			Gelatin mixes		
Time	Temp.	Overrun	Time	Temp.	Overrun
Mix 7					
min.	°*F.*	*pct.*	*min.*	°*F.*	*pct.*
0	52.6	0	0	47.5	0
1	30.3	37	1	28.6	33
2	27.0	53	2	26.7	38
3	26.0	60	3	25.4	50
4	25.4	64	4	24.7	58
5	24.7	74	5	24.3	72
6	24.7	95	6	24.4	97
7	24.9	109	7	24.7	111
8	25.1	120	8	25.0	117
9	25.4	124	9	25.2	120
10	25.7	130 [1]	10	25.5	128 [1]

Part 2—10-quart counter freezer

Mix	Time to reach 100% overrun		Temperature at 100% overrun	
	Sodium alginate	Gelatin	Sodium alginate	Gelatin
	min.	*min.*	°*F.*	°*F.*
1	11.0	12.0	25.3	25.4
2	10.0	11.0	25.2	25.2
3	10.0	11.0	25.1	25.4
4	9.5	11.0	24.8	25.5
5	9.5	10.0	25.1	25.4
6	9.5	10.5	.....	25.4
7	17.0 [2] (134%)	17.0 [2] (138%)	26.4	26.4

[1] Maximum. [2] Mix whipped until maximum overrun was obtained.

with calcium, would show relatively better whipping than when stabilized with gelatin. This would be especially noticeable in freezers that are slow whippers.

Samples of the ice cream containing gelatin and sodium alginate at 100 per cent overrun and at the maximum overrun obtainable were compared but little difference was detectable in body and the samples were scored the same by the authors. Eight experienced judges were asked to assist in the judging, with the following results:

Set	Sample	Number placing first	Number placing second	No choice
1—100% overrun	Sodium alginate	1	7	0
1—100% overrun	Gelatin	7	1	0
2—130% overrun	Sodium alginate	5	3	0
2—128% overrun	Gelatin	3	5	0
3—100% overrun	Sodium alginate	1	3	3
3—100% overrun	Gelatin	3	1	3

Sets 1 and 2 were frozen in a 50-quart batch freezer while Set 3 was frozen in a 10-quart counter freezer.

Comparison of Stabilizers in Butter Mixes: Since factors affecting the whipping quality of mixes are likely to be more apparent in butter mixes than in cream mixes, experimental lots were prepared in which butter was the entire source of fat. The mixes were standardized to contain 12 per cent fat, 11.5 per cent serum solids, and 15 per cent sugar. Thirty-five hundredths of one per cent gelatin was used in one batch and .3 per cent sodium alginate was used in the other batch. All ingredients were added before homogenization. After aging 24 hours, the gelatin mix had a viscosity of 68 at 10°C. (50°F.) and the sodium-alginate mix had a viscosity of 83. One batch of the gelatin mix whipped to 102 per cent overrun in eight minutes, and the second batch whipped to 100 per cent overrun in eight minutes. One batch of the sodium-alginate mix whipped to

TABLE 4

Color Analyses of Mixes Containing Sodium Alginate and Gelatin

Wave length (millimicrons)	Photometric readings			
	.35% 225 Bloom gelatin	.3% sodium alginate	.35% sodium alginate	.40% sodium alginate
480	55	54	52	52
510	60	59	58	58
540	65	65	65	65
570	69	67	65	65
600	68	67	65	65
630	69	68	65	65

98 per cent overrun in eight minutes, and the other batch whipped to 98 per cent overrun in seven minutes. The body scores of the ice creams were identical.

Effect of Sodium Alginate Upon Color of Mix: In the use of the Keuffel and Esser color analyzer the color reflection of the unknown is compared with that of magnesium carbonate as a standard with a reading of 100. The lower the readings obtained the more absorption of light that has occurred or the darker the substance. By referring to the data (Table 4) it will be seen that in general the mixes containing sodium alginate were slightly darker in color than the gelatin mixes. It might be added, however, that this difference is so slight it is hardly perceptible to the eye. Peculiarly, in the case of the frozen ice creams the product stabilized with sodium alginate seems to have less color than that containing gelatin.

Acidity of Mix Affected by Sodium Alginate: It was noticed that the mixes containing the sodium alginate always had a lower titratable acidity than those containing gelatin. This is due to the alkaline reaction that the sodium alginate has (Table 5).

SUMMARY

Certain properties of sodium alginate as a stabilizer in ice cream have been studied and compared with those of gelatin.

The most practicable method of adding sodium alginate to the mix is to add it dry mixed with sugar, or dispersed in water, when the mix temperature has reached 71.1°C.(160°F.) but before homogenization.

Owing to its chemical affinity for calcium, the action of sodium alginate upon mix viscosity is almost immediate, while that of gelatin is a function of time as well as temperature. It is important, therefore, to avoid using an excessive amount of the sodium alginate in order not to delay mix cooling and pumping operations.

TABLE 5

Effect of Sodium Alginate on Mix Acidity

Mix and amount of stabilizer	Titratable acidity	Hydrogen-ion concentration
	pct.	
.35% 225 Bloom gelatin	.20	6.50
.30% sodium alginate	.18	6.61
.35% sodium alginate	.16	6.70
.40% sodium alginate	.15	6.79

Although a Keuffel and Esser color analyzer will show slightly more color in a mix stabilized with sodium alginate, the ice cream appears upon freezing to have less color.

Sodium alginate has a slight alkaline reaction in the mix and will cause a lowering of the percentage of titratable acidity and a decrease in the hydrogen ion concentration.

The relative efficiency of sodium alginate and gelatin as stabilizers of ice cream, as measured by their effect upon the whipping qualities of the mix and the body of the ice cream, is dependent upon the conditions of the comparison. Variable conditions under which different investigators have worked, particularly the mineral content of the mix and the kind of freezers used, possibly account for some of the differences in results obtained. The data herein presented indicate that for a 12-per cent mix a sodium-alginate (Dariloid) content of about .3 per cent should be used. This amount of the stabilizer will give results that will approximate those obtained with .35 per cent of a 225 Bloom gelatin. In mixes containing higher solids the amount of these two stabilizers will naturally need to be reduced proportionately.

The possibility of the introduction of new types of sodium-alginate stabilizers, however, makes such comparison of little value. It is not

at all unlikely that sodium alginate of double or triple the strength of Dariloid may be made available for the ice cream industry. Possible variations in the strength of the gelatin used also makes it impossible to establish a definite relative relationship between sodium alginate and gelatin in general.

When using the above-recommended amounts of sodium alginate and gelatin there will be no decided preference for either stabilizer so far as the body of the ice cream or whipping time is concerned. While data averages were somewhat more favorable for the gelatin, the differences were too slight to justify definite conclusions to the effect that the gelatin was better. In a limited number of trials somewhat shorter whipping periods resulted from the use of the sodium alginate when using a counter freezer, suggesting that with certain freezers sodium alginate might give more favorable results than would gelatin.

The melt-down characteristics of ice cream stabilized with sodium alginate and gelatin are somewhat different. The former ice creams melt more rapidly when exposed to room temperature and have a smoother appearance than the latter.

No differences in flavor were detectable, and in no case was an off-flavor present in the ice cream that could be attributed to the stabilizer used.

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MINERAL CONSTITUENTS OF HONEY. IV. SODIUM AND POTASSIUM [1]

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The number of communications which contain data relevant to this phase of the subject of mineral constituents of honey is probably not more than eight. The list begins with a report by Thompson (1908) on the composition of the algaroba honey of the Hawaiian Islands, potassium alone of the alkali metals being accounted for. Interest in this subject soon thereafter shifted to Europe as the comparative analyses by Fehlmann (1912) of honeys representing a larger number of floral sources testify. Following an interlude of some 15 years came a revival of interest there in the subject through the researches of Nottbohm (1927); of Elser (1928), who determined only the potassium content; of Sundberg and Lundgren (1930); and of Svoboda (1930, 1933). Within the past decade, also, Australia entered the picture through the publication by Jewell (1931) of the results of his analysis of five native honeys. Honeys grown in continental United States to date have not received similar attention.

The results of a survey which was made to fill this gap are herein recorded. Included among the 31 samples obtained for this purpose from different areas of the United States were various clover honeys from the central and eastern states, sage and orange blossom honeys from California, alfalfa honeys from the mountain states, buckwheat honeys from Wisconsin and New York, and five typical of the Georgia crop. Every one of seven commercial color grades, from water-white through extra-light amber to dark was represented in the group assembled for this study.

EXPERIMENTAL PROCEDURE

Inasmuch as the problem of accurately determining the alkali metal content, particularly sodium, in the product of a wet digestion presents some difficulties not only because of a high sodium blank in

[1] This paper is the fourth of a series called "Mineral Constituents of Honey." The first three papers in the series have been published as follows:

1. Schuette, H. A., and Remy, K., 1932. Degree of pigmentation and its probable relationship to the mineral constituents of honey. J. Am. Chem. Soc. **54**, 2909.
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those reagents which are suitable for oxidizing strongly saccharine materials but also because of the solvent action of the former upon glassware, a dry-ashing technique was used throughout this study. Incineration of the carbonized residues of 50-gram samples were made in platinum dishes kept for eight hours in an electrically heated furnace adjusted for a temperature below 500°C.(932°F.). Recovery experiments had shown that under these conditions no potassium losses need be feared.

The elements in question were determined gravimetrically by methods which, by preliminary experiment and recovery trials, proved to be simple, rapid, accurate, and adaptable to the problem

TABLE 1

Ash, Sodium, and Potassium Contents of Honey

Classification	Samples	Ash	Sodium		Potassium	
			Pct. of ash	Mg. per kg.	Pct. of ash	Mg. per kg.
Light honeys		*pct.*				
Water-white	3	.04	4.13	17	28.1	117
White	10	.10	3.05	19	42.6	293
Average	....	.07	3.59	18	35.3	205
Dark honeys						
Light amber	9	.12	2.99	27	37.1	467
Amber	3	.22	4.92	31	37.2	769
Dark	6	.78	1.45	94	24.7	2117
Average	....	.56	4.68	76	33.0	1676

in hand. For the estimation of potassium the Winkel-Maas (1936) dipicrylamine procedure was used; for sodium the Barber-Kolthoff (1929) zinc uranyl acetate procedure served.

RESULTS

Consistent with the practice followed in previous communications from this laboratory on the subject of the inorganic constituents of honey, data are recorded by color groups according to position on the Pfund scale (Table 1) rather than individuals. In the light-honey group are placed the water-white and the white honeys; in the deeper pigmented group are placed the light amber, the amber, and the dark honeys. The effect of this division is that the first group consists of honeys whose predominating floral sources are the various clovers, orange blossoms, cotton, and white sage; the second consists of buckwheat, wild aster, and other late-blooming flowers and representatives of southern nectar sources, such as gallberry, tupelo, titi, Mexican clover, and thistle. Since the compilation herein made does not reveal

the limits within which sodium and potassium occur in each main division, the variations in content thereof have been made the subject of a separate one (Table 2).

DISCUSSION

With the completion of this study of the sodium and potassium contents of 31 honeys which had been produced exclusively in the United States there is now afforded opportunity for making comparison with similar data relevant to honeys of other geographical origins (Table 3). It is perhaps unfortunate that more data are not available on honeys grown elsewhere than in the United States—the whole number involved is approximately the same as examined in this study—for then more satisfactory comparisons, based on geographical distribution of product, could be made than the present survey admits. With these limitations, then, it appears that the Australian honeys examined by Jewell (1931) contained more sodium but less potassium than those reported on herein, and that the European group falls below the American in both respects.

TABLE 2

Variation in Sodium and Potassium Contents of Light and Dark Floral Honeys

Classification	Sodium		Potassium	
	Pct. of ash	Mg. per kg.	Pct. of ash	Mg. per kg.
Light honeys				
Minimum	0.96	6	23.0	100
Maximum	9.26	35	70.8	588
Dark honeys				
Minimum	0.20	9	2.0	115
Maximum	11.20	400	61.6	4733

It is apparent here, too, that potassium predominates over sodium as is the case in practically all other natural foods. With increasing degree of pigmentation there appears to come a larger content of the alkali metals, the differential between the major color groupings set up here being larger with respect to potassium than to sodium. In the light of our present knowledge of the subject, however, whatever relationship may exist here must be deemed to have a qualitative, rather than a quantitative, significance.

SUMMARY

That there probably exists a qualitative relationship between degree of pigmentation as measured by the current practice of color-

grading this food and the quantity of sodium and potassium which it contains, appears to be a permissible conclusion upon analyzing the data obtained in the examination of 31 honeys deemed to include representatives of most of the honeys produced commercially in the United States. Viewed in the light of the data recorded in this report and in others of this series on the mineral constituents of honey, it appears, also, that potassium is the predominating constituent of the ash. The following minima and maxima were found for the Ameri-

TABLE 3

Classified Summary of Sodium and Potassium Contents of Floral Honeys Reported During 1908-1938

Origin	Sodium		Potassium	
	Mg. per kg.	Pct. of ash	Mg. per kg.	Pct. of ash
Australia	37-765[5*]	0.74-3.4[5]	548-1785[5]	23.2-33.5[5]
Europe				
Austria	.....	.....	416-1768[4]	.....
Czechoslovakia	.....	1.37[1]	.....	44.7[1]
Germany	.....	2.34-7.46[3]	.....	31.7-47.4[3]
Russia	.....	2.33[1]	.....	44.70[1]
Sweden	36-133[6]	1.81-7.32[6]	318-2365[6]	35.7-46.6[6]
Switzerland	.....	2.98-15.70[11]	.....	32.4-50.5[11]
Unclassified	.....	5.33-6.68[2]	.....	24.9-32.1[2]
North America				
Hawaii	.....	4.12[1]	.....	45.0
United States	6-400[31]	1.17-112[31]	100-4733[31]	18.5-70.8[31]

* Superior numbers indicate the number of samples involved in this survey.

can honeys examined: sodium, 6 and 400 milligrams per kilogram, respectively, or .96 and 11.2 grams per 100 grams of ash; potassium, 100 and 4,733 milligrams per kilogram, respectively, or 18.5 and 70.8 grams per 100 grams of ash. All available data on this phase of honey composition have been collected and collated.

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IODINE IN CITY WATERS AND VEGETABLES IN TEXAS

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The relation between the prevalence of goiter and a low supply of iodine in the food and water supplies in a given area has been shown by the work of a large number of investigators. Marine, Lenhart, and Kimball (1923) demonstrated that iodine at the rate of 300 milligrams per year is effective in the control of a large percentage of simple goiter. The minimum quantity of iodine required per day has been estimated by Meerburg (1935) to be from 80 to 100 micrograms, by Cameron (1931) from 35 to 70 micrograms, and by Orr and Leitch (1929) about 45 micrograms for an adult male and 150 micrograms for a child. Levine and Remington (1933) and others have shown that the amount of iodine required by an individual is determined to some extent by a number of factors other than the actual quantity of iodine ingested. These include the quantity of lime, vitamins, and such foods as cabbage and fats in the diet; and also climatic factors, such as light, temperature, and seasonal variation.

Weston (1933) has suggested that a complete survey of the iodine question in all areas of the country be made. Such a survey was begun by the writers in Texas a number of years ago, in order to provide a basis for estimating whether or not sufficient iodine for proper development and maintenance was being ingested by Texans in their regular supplies of drinking water and vegetables. The iodine content of Texas vegetables is also of considerable general interest since large quantities of vegetables produced in Texas go into the great food markets in other states. This paper presents the results of the study of iodine content of city waters and vegetables from a number of sections of Texas.

EXPERIMENTAL PROCEDURE

Water samples were collected in gallon bottles and brought or sent to the laboratory. An aliquot, usually 500 c.c., was evaporated to about 30 c.c. in the presence of a slight excess of sodium hydroxide. Iodine was then estimated by the method described below.

Vegetables to be analyzed were cut into small pieces and dried in an oven at about 45°C.(113°F.). After drying, the material was ground in a Wiley mill and stored in tightly closed jars. When the analysis was made, a portion of the sample, varying from 25 to 100

grams, was burned in a current of oxygen in the apparatus described by von Kolnitz and Remington (1933). The combined absorbing solution and washings were then evaporated to about 30 c.c. and iodine estimated by the method described below.

The estimation of iodine was made by a colorimetric modification of the method described by Trevorrow and Fashena (1935) and improved by Fashena and Trevorrow (1936). The oxidation of iodine and residual organic matter by chromic acid, reduction of iodates by phosphorous acid, and distillation in an all-glass apparatus were conducted as they describe, with the exception that the iodine was absorbed in .02 N sodium hydroxide. The distillate containing the iodine was acidified slightly, using bromphenol blue as an outside indicator, and one drop of sulfuric acid (one part acid to one part water) added in excess. Bromine vapor was then added to the solution to a brown color. The solution was then boiled to a volume of about 5 c.c., cooled, and transferred to a 30-c.c. separatory funnel marked at 10 c.c. The beaker was washed with very small quantities of water and the washings added to the funnel to a volume of 10 c.c. Four drops of sulfuric acid, one c.c. of .1 per cent potassium iodide solution, and one c.c. of carbon tetrachloride were then added in the order given, with shaking between each addition. The solution was then shaken vigorously for two minutes. The carbon tetrachloride was run out into a 15-c.c. centrifuge tube, and centrifuged at high speed for one minute. The color was compared in a Duboscq microcolorimeter with a standard prepared in the same way.

WATER ANALYSES

With a very few exceptions, all of the samples of water from the cities of Texas from which samples were secured are comparatively well supplied with iodine. The mean iodine content of all of the samples was 56 parts per billion, with a standard deviation of 60 parts per billion. The average iodine content for the various regions (Table 1) shows that waters from the East Texas Timber country and the West Cross Timbers regions are considerably lower in iodine than those from the other regions of the state. Both of these regions are covered with sandy, noncalcareous soils. The East Texas Timber country is in the humid section of the state, while the West Cross Timbers are on the boundary between the humid and subhumid sections. The highest average quantities of iodine are found in waters from the high plains and the Rio Grande Plain. Both of these regions are covered, in the main, with heavier soils, and both are in the subhumid section of the state. Waters containing low quantities of iodine occur throughout the state, however, as

is shown by the mean value minus the standard deviation and the minimum analyses (Table 1). Rain water, collected in cisterns, is used for cooking and drinking in many parts of the state, and rain water is stored in earthen "tanks" for the use of animals. These must be much lower in iodine than the samples examined.

The relation between the iodine content of a sample of water and other salts in the water is of interest. Waters throughout the Middle West, where the incidence of goiter is supposed to be relatively high, are in most cases well supplied with calcium salts but low in sodium salts. Many of the samples from Texas contain considerable quantities of sodium salts in addition to those of calcium. The coefficient of correlation between iodine and calcium salts was .0335, while that for iodine and sodium salts was .4099. The coefficient of correlation with calcium salts is not significant, while that for sodium salts is highly significant. The principal calcium salt is calcium carbonate, while the principal sodium salt is sodium chloride. The coefficient of correlation between iodine and calcium carbonate was .1007 and is not significant, while that between iodine and sodium chloride was .8131 and is highly significant.

When compared with waters from some other regions, the city waters of Texas are high in iodine. Adolph and Prochaska (1929) report the iodine content of water from seven wells in Nebraska as containing from .5 to 30 parts per hundred billion. Szniolis and Marcinkowska-Lapienska (1935) report that 56 samples of water in Poland contained from 46 parts per billion in northern Poland, where no goiter occurs, to .05 part per billion in the southern mountainous region, where frequent goiter cases occur. Lunde (1936) reports that the drinking water from a goiter region in Norway contained from 1.5 to 2 parts per billion of iodine. These figures are considerably lower than those for most of the samples of Texas waters.

Very few of the samples of water examined in this study can be considered deficient in iodine. As noted above, the average requirement of iodine per day is estimated by many investigators to be about 100 micrograms. The mean iodine content of 103 samples of Texas water was 56 micrograms per liter. Two quarts of average water would thus contain sufficient iodine for a day's requirement, without any supplementing by the iodine in the food consumed. Most people in Texas probably drink considerably more than that quantity per day and, in so doing, ingest in their drinking water alone sufficient iodine for proper development and maintenance.

VEGETABLE ANALYSES

Vegetables were secured from Weslaco in the Rio Grande Valley, Winter Haven in the western section of the Rio Grande Plain,

TABLE 1

Average Iodine[1] in City Waters in Regions of Texas

Region	Total samples examined	Number of samples						Iodine content		
		Iodine by groups								
		0-10	11-25	26-50	51-75	76-100	101+	Mean	Standard deviation	Minimum
East Texas Timber Country	33	4	15	14	0	0	0	24	12	9
West Cross Timbers	4	0	3	0	1	0	0	28	16	14
Rolling Plains	13	0	5	6	1	0	1	38	27	13
Grand Prairie	1	0	0	1	0	0	0	43	....	43
Basins and mountains	1	0	0	1	0	0	0	48	....	48
Edwards Plateau	3	0	0	1	2	0	0	55	11	49
Gulf Coast Prairie	5	0	1	2	0	1	1	58	37	25
Blackland Prairies	20	0	3	5	7	1	4	68	49	15
Rio Grande Plains	14	0	3	3	2	0	6	99	89	14
High Plains	9	1	1	1	1	1	4	114	101	4
All regions	103	5	31	34	14	3	16	56	60	4

[1] Expressed in parts per billion.

Sugarland and Prairie View on the Gulf Coast Prairie, and College Station in the East Texas Timber Country. While most of the vegetables were high in iodine, the content of the same vegetable varied considerably with the portion of the plant used for food, variety, location, and soil (Table 2). The leafy vegetables contained a great deal more iodine than root or fruit vegetables. Greens from beets and turnips contained about 75 per cent more iodine than roots from the same plants. Copenhagen Market cabbage from Weslaco contained over four times as much iodine as All Head cabbage collected at the same time and place. Vegetables from College Station contained 59 per cent more iodine than corresponding vegetables grown at Weslaco and about 25 per cent more than those from Sugarland. A sample of Italian Green Sprouting broccoli grown on a Brennan fine sandy loam at Weslaco contained over eight times as much iodine as the same vegetable grown on a Duval fine sandy loam at Winter Haven. Notwithstanding considerable variation in the iodine content with different factors involved in the growth of the vegetables, very few of the vegetables analyzed can be considered deficient in iodine.

The vegetables analyzed contained quantities of iodine which are as high as, or higher than, amounts reported for similar vegetables from other states (Table 3). In addition to the results listed (Table 3), Remington, Culp, and von Kolnitz (1929) report that potatoes from Michigan contained 94 parts per billion of iodine and potatoes from Minnesota, 86 parts per billion; while Frear (1934) reported an average of 78 parts per billion in potatoes grown in Pennsylvania. Irish potatoes grown in Texas thus contained a great deal more iodine than potatoes grown in California, Michigan, Minnesota, and Pennsylvania. Texas vegetables are in general comparatively high in iodine.

SUMMARY

Waters from all of the Texas cities studied are well supplied with iodine. The iodine content is usually highest in geographical regions of the state which are covered with relatively heavy soils and are in the subhumid section of the state. The quantity of iodine in waters was not significantly correlated with calcium salts nor with calcium carbonate, but was significantly correlated with sodium salts and sodium chloride.

Vegetables grown in Texas are high in iodine. The iodine content of leafy vegetables is much higher than that of root or fruit vegetables. The iodine content of vegetables varied greatly with the variety, location, and soil. Vegetables grown in Texas are as high in iodine

TABLE 2

Iodine in Vegetables Grown in Texas

Vegetable	Variety	Location	Soil type	Iodine
				p.p.b.
Beans	Bountiful	Weslaco	Victoria sandy loam	955
Beets, leaves	Unknown	Sugar Land	Miller silt loam	915
Beets, leaves	Unknown	Prairie View	Hockley fine sandy loam	702
Beets, leaves	Unknown	College Station	Lufkin fine sandy loam	369
Beets, leaves	Dark Red Turnip	College Station	Lufkin fine sandy loam	1,030
Beets, roots	Unknown	Sugar Land	Miller silt loam	588
Beets, roots	Unknown	Prairie View	Hockley fine sandy loam	431
Beets, roots	Unknown	College Station	Lufkin fine sandy loam	180
Beets, roots	Dark Red Turnip	College Station	Lufkin fine sandy loam	308
Broccoli	Italian Green Sprouting	Winter Haven	Duval fine sandy loam	429
Broccoli	Italian Green Sprouting	Weslaco	Brennan fine sandy loam	3,502
Broccoli	Glory of Calabria	Weslaco	Brennan fine sandy loam	521
Brussels sprouts	Long Island Improved	Winter Haven	Duval fine sandy loam	62
Cabbage	Unknown	Sugar Land	Miller silt loam	1,208
Cabbage	Marion Market	Sugar Land	Miller silt loam	2,163
Cabbage	Enthuizen	Weslaco	Victoria sandy loam	370
Cabbage	All Head	Weslaco	Victoria fine sandy loam	153
Cabbage	Copenhagen Market	Weslaco	Victoria fine sandy loam	667
Carrots	Danver's	Weslaco	Victoria fine sandy loam	233
Eggplant	Imperial Black Beauty	Weslaco	Webb fine sandy loam	112
Endive	Green Curled	Weslaco	Brennan fine sandy loam	559
Mustard	Southern Giant Curled	Weslaco	Brennan fine sandy loam	498
Mustard	Tendergreen	Weslaco	Webb fine sandy loam	1,071
Mustard	Unknown	Sugar Land	Miller silt loam	510
Mustard	Southern Giant Curled	Weslaco	Victoria fine sandy loam	866
Mustard	Southern Giant Curled	College Station	Lufkin fine sandy loam	1,185
Okra	White Velvet	Weslaco	Webb fine sandy loam	251
Peppers	California Wonder	Weslaco	Webb fine sandy loam	312
Potatoes, Irish	Early Triumph	Sugar Land	Miller silt loam	220
Potatoes, Irish	Bliss Triumph	Weslaco	Victoria sandy loam	347
Potatoes, Irish	Triumph	Weslaco	Victoria fine sandy loam	162
Potatoes, Irish	Irish Cobbler	Sugar Land	Lake Charles Clay loam	160
Potatoes, Irish	Triumph	Sugar Land	Lake Charles Clay loam	1,045
Spinach	Unknown	Sugar Land	Miller silt loam	821
Spinach	Bloomsdale Savoy	Weslaco	Brennan fine sandy loam	679
Squash	Giant Summer Straightener	Weslaco	Webb fine sandy loam	290
Swiss chard	Giant Lucullus	College Station	Lufkin fine sandy loam	2,088
Tomatoes	Bonny Best	Weslaco	Webb fine sandy loam	351
Tomatoes	Marglobe	Weslaco	Brennan fine sandy loam	339
Turnips, leaves	Shogoin	Weslaco	Webb fine sandy loam	723
Turnips, leaves	Purple Top White Globe	College Station	Lufkin fine sandy loam	1,525
Turnips, roots	Shogoin	Weslaco	Webb fine sandy loam	460
Turnips, roots	Purple Top Globe	Weslaco	Victoria fine sandy loam	705
Turnips, roots	Purple Top White Globe	College Station	Lufkin fine sandy loam	833

as those grown in other states for which high iodine analyses have been reported. Iodine in Irish potatoes grown in Texas was much higher than in potatoes grown in California or the Middle West.

TABLE 3

A Comparison of Iodine[1] in Vegetables From Different States

	Texas	Okla.[2]	Ga.[3]	S. Car.[4]	Cal.[4]
Beans	955	501	.....	.....	.....
Beets, roots	377	245	.....	.....	.....
Beets, leaves	854	651	.....	.....	8
Cabbage	912	286	.....	.....	.....
Carrots	233	507	.....	.....	8
Mustard	826	561	.....	.....	.....
Okra	251	777	.....	412	.....
Potatoes	387	210	54	333	.....
Spinach	750	483	567	.....	32
Tomatoes	345	379	.....	.....	20
Turnip greens	1,124	676	226	.....	.....

[1] Expressed in parts per billion. [2] Heller, Jones, and Pursell (1935). [3] Holley, Pickett, and Brown (1935). [4] McClendon and Remington (1929).

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HYDROLYTIC CLEAVAGE AND OXIDATION OF SOYBEAN MEAL BY *PENICILLIUM LUTEUM PURPUROGENUM* AND *ASPERGILLUS NIGER*

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Most of the chemical changes produced by microörganisms are said to be due to the activity of catalysts produced directly or indirectly by the cell. The ability of certain molds to hydrolyze and in turn oxidize carbohydrates has been investigated. Glucose, for instance, can be converted into alcohol, citric, gluconic, and oxalic acids as well as numerous other products, depending upon the conditions and organisms employed. May, *et al.* (1929) reported a semi-plant scale process for the production of gluconic acid by the action of *Penicillium luteum purpurogenum* on solutions of commercial dextrose. Yields as high as 60 per cent of the theoretical of gluconic acid were obtained, May, *et al.* (1927). Other investigators have identified proteoses in molds. Bourquelot and Herissey (1898) noted the hydrolysis of casein by *Aspergillus Niger*. Malfitano (1900) found a protease in this organism which could be isolated in acid solution.

Soybean meal contains both carbohydrates and proteins. It was of interest, therefore, to investigate which part and to what extent each part of the meal was attacked by these organisms. Would the carbohydrate in the meal be acted upon even in presence of a high nitrogen content? Are there any changes in the metabolic adaptations of the organism; that is, which part of the meal is acted upon to a greater extent with the change in the age of the culture?

EXPERIMENTAL PROCEDURE

Soybean meal of three different granulations, fine, medium, and coarse, was furnished by the Glidden Company, Chicago. The chemical analysis of the meal is shown (Table 1) and chemical analysis of the ash (Table 2).

Preparation of the Substrate: Forty grams of soybean meal—medium mesh was found to be best—were mixed with 800 c.c. of distilled water in a two-liter Erlenmeyer flask, stoppered with cotton, and then sterilized at 20 pounds pressure for one-half hour. Forty c.c. of the liquid were then withdrawn by use of sterile pipettes and

titrated with N/50 sodium hydroxide to see if any hydrolysis took place during autoclaving (Table 3).

Inoculation: The cultures used were *Penicillium luteum purpurogenum* (American Type Culture Collection 1934 catalog No. 4713). They were grown on agar slants. Cultures ten days old, or older, were placed on the surface of the liquid. (Each time the culture submerged it was found that no growth took place.)

TABLE 1

Chemical Analysis of Soybean Meal

	Fine mesh	Medium mesh	Coarse mesh
	pct.	*pct.*	*pct.*
Reducing sugars	1.73	2.45	2.09
Non-reducing sugars	8.90	10.91	12.03
Crude protein	45.30	45.90	46.75
Albumoid nitrogen	44.70	46.50	42.80
Ash and minerals	4.95 (ash)	5.11 (ash)	5.03 (ash)

Fermentation: A temperature between 23 to 26°C. (73.4 to 78.8°F.) was chosen because of the optimum yield of gluconic acid at that temperature reported by Herrick and May (1928). Growth took place very slowly for the first three or four days. After 30 days the surface was completely covered. At that time the liquid in

TABLE 2

Analysis of the Ash

Chemical element	Amount
CaO	4.5
MgO	6.5
P_2O_5	30.5
SO_3	3.0
Cl	0.75 - 0.8
K_2O	By difference about
Na_2O	50.0

the flask was strained through muslin and then filtered. Forty c.c. of this filtrate were titrated with N/50 sodium hydroxide (Table 3), using phenolphthalein as indicator.

These results clearly indicate, as might be expected, that practically no hydrolysis and oxidation of the carbohydrate has taken place because of the high nitrogen content of the substrate, May, *et al.* (1929). The protein portion of the meal was attacked, however, as shown (Table 3). To the same 40 c.c. portion, as mentioned above after neutralization with N/50 sodium hydroxide, 25 c.c. of

neutralized formaldehyde were added and titrated again with N/50 sodium hydroxide (Sorensen's titration).

The biuret reaction was positive in each case. Tests for some free amino acids were also carried out but none were found to be present. Tyrosin, Deniges (1900a, 1900b) and Folin and Denis (1912); leucine, Wurster (1888); and phenylalanin, Mayeda (1907) were tested for. Dakin's (1920) butyl alcohol extraction method for the isolation of mono-amino, monocarboxylic acids was used, but it yielded none of them.

TABLE 3

Sorenson Titration on Substrate After Inoculation With Penicillium Luteum Purpurogenum[1]

Flask No.	N/50 Na (OH) added		
	After sterilization at 20 lbs. pressure for one-half hour	30 days after inoculation with *Penicillium luteum purpurogenum*	
		Filtrate	Protein
	c.c.	*c.c.*	*c.c.*
1	.45	1.40	23.00
2	.44	1.15	28.80
3	.50	2.15	30.40
4	.45	1.30	22.40
5	.60	3.25	23.50
6	.43	1.75	23.60
7	.40	1.96	23.40
8	.50	Spoiled	Spoiled
9	.45	0.75	24.70
10	.48	1.25	23.95
11	.55	1.75	23.50
12	.45	1.65	23.60
13	.53	1.95	24.10
14	.48	1.85	22.70

[1] The titration after sterilization was carried out to see if any hydrolysis of the protein took place during autoclaving.

DISCUSSION OF RESULTS

Penicillium luteum purpurogenum under the conditions employed did not attack the carbohydrate portion of the soybean meal to any appreciable extent, and if any acid was formed the amount was so small that it could be neither isolated nor detected.

The organism did attack the protein portion of the meal, hydrolyzing it partially.

Hydrolysis did not go all the way to the free amino-acid stage. The reaction products in solution still gave a biuret test.

EXPERIMENT USING *ASPERGILLUS NIGER*

This organism (American Type Culture Collection 1934 catalog No. 1015) was also grown on agar slants for a period of six days

TABLE 4

Sorenson Titration on Substrate After Inoculation With Aspergillus Niger

Age of cultures	Flask No.	NaOH N/50 after sterilization	NaOH N/50 after inoculation	NaOH N/50 after addition of 25 c.c.HCHO	In solution until titrated
days					*days*
6	1	2.00	17.10	40.50	15
6	2	1.90	18.05	39.80	15
6	3	2.10	24.10	67.20	18
6	4	1.85	25.20	68.10	18
6	5	2.10	29.40	89.80	21
6	6	1.75	28.45	90.50	21
6	7	1.60	28.20	93.50	27
6	8	1.90	30.20	91.10	27
6	9	1.75	31.20	95.05	30
6	10	1.65	32.30	94.45	30
10	11	1.80	20.10	40.60	15
10	12	1.90	20.30	41.20	15
10	13	1.90	27.40	59.10	18
10	14	2.10	28.10	60.50	18
10	15	1.80	32.10	69.80	21
10	16	2.05	33.20	70.40	21
10	17	1.70	48.50	75.10	27
10	18	1.80	49.10	76.05	27
10	19	1.65	60.20	87.60	30
10	20	2.10	61.10	87.50	30
20	21	1.70	34.80	23.30	12
20	22	2.40	33.40	24.05	12
20	23	1.90	47.10	39.10	15
20	24	2.05	47.50	40.50	15
20	25	1.85	66.20	43.20	21
20	26	1.70	63.20	42.80	21
20	27	1.80	70.45	46.90	25
20	28	1.90	69.10	45.80	25
20	29	1.65	80.35	48.50	27
20	30	2.05	81.40	49.20	27
20	31	1.60	82.90	54.00	28
20	32	1.70	83.40	55.10	28
20	33	1.85	90.30	63.45	32
20	34	1.75	91.10	64.50	32
20	35	2.00	105.90	67.55	33
20	36	1.90	103.10	68.60	33
20	37	1.85	112.05	86.55	39
20	38	1.75	111.10	85.40	39
33	39	1.65	66.40	60.10	21
33	40	1.81	65.10	60.70	21
63	41	1.70	78.10	69.80	22
63	42	1.83	77.80	68.70	22
113	43	1.90	71.40	46.70	25
113	44	1.60	72.30	47.20	25
113	45	1.75	82.90	54.20	27
113	46	1.80	83.20	54.80	27
113	47	1.95	90.70	64.30	32
113	48	1.75	92.10	65.40	32
113	49	2.10	102.10	68.20	33
113	50	1.90	101.60	67.90	33
113	51	1.80	113.70	87.20	39
113	52	1.75	114.50	88.10	39

or more and then added to the substrate, which was prepared in the same manner as for the previous experiment. The cultures grew much faster and at the end of 10 to 15 days the whole surface of the liquid was covered. The contents of the flasks were strained and filtered and 40 c.c. of the filtrate were titrated with N/50 sodium hydroxide (Table 4).

Tests for free amino acids as well as attempts to isolate them were negative. Gluconic and citric acids could not be isolated.

TABLE 5

Sorenson Titration on Substrate After Inoculation With Aspergillus Niger Grown on Agar Slants Containing Vitamin B_1

Age of cultures	Flask No.	NaOH N/50 after sterilization	NaOH N/50 after incubation	NaOH N/50 after addition of 25 c.c. HCHO	Standing until titration
days					*days*
6	1	1.80	18.2	42.1	15
6	2	2.00	16.1	40.8	15
6	3	1.70	30.1	88.2	21
6	4	1.65	29.2	89.5	21
6	5	1.60	32.4	93.9	30
6	6	1.85	33.7	94.7	30
10	7	1.80	20.7	41.0	14
10	8	1.90	21.1	41.2	14
10	9	1.70	33.4	70.3	22
10	10	1.75	33.8	71.4	22
10	11	1.80	62.1	89.1	30
10	12	1.60	61.9	88.3	30
20	13	2.00	48.3	42.1	15
20	14	1.75	47.9	41.3	15
20	15	1.85	67.4	46.5	23
20	16	1.80	68.2	46.7	23
20	17	1.70	86.7	57.2	30
20	18	1.75	87.1	58.4	30
20	19	1.65	108.4	83.2	38
20	20	1.90	110.1	83.9	38

In order to estimate the effect of vitamin B_1 on the fermentation by *Aspergillus Niger* on the soybean meal, two different procedures were followed:

a. The organism was grown on agar slants containing about one milligram per liter of Betabion (B_1 HCl, Merck). Growth on the slants took place much faster, but when added to the substrate, results similar to those in the preceding experiment were obtained (Table 5).

b. When the vitamin B_1 was added to the substrate after sterilization—to prevent destruction— (.8 milligram per 800 c.c.)

different results were obtained (Table 6). While the "percentage yields" of acids were substantially the same as in the experiments in which no growth stimulant was present, the rate of fermentation was more rapid.

TABLE 6

Sorenson Titration on Substrate After Inoculation With Aspergillus Niger With Vitamin B_1 Added to Substrate

Age of cultures	Flask No.	NaOH N/50 after sterilization	NaOH N/50 after incubation	NaOH N/50 after addition of 25 c.c. HCHO	Standing until titration
days					*days*
6	1	1.80	21.30	43.1	15
6	2	1.70	22.10	42.5	15
6	3	1.95	34.10	92.1	20
6	4	1.85	33.40	93.2	20
10	5	2.10	25.10	43.1	15
10	6	2.00	25.90	44.2	15
10	7	1.80	35.10	73.4	20
10	8	1.90	35.60	74.1	20
10	9	1.90	59.30	87.2	25
10	10	1.75	59.80	87.6	25
20	11	1.80	40.10	30.7	12
20	12	1.65	40.80	30.2	12
20	13	1.75	53.60	41.2	15
20	14	1.90	54.20	41.6	15
20	15	1.70	67.80	44.5	20
20	16	2.00	68.20	45.1	20
20	17	1.90	80.35	48.9	25
20	18	1.85	81.40	49.3	25
20	19	1.65	91.20	63.7	28
20	20	2.00	91.60	64.5	28

CONCLUSIONS

1. *Aspergillus Niger,* unlike *Penicillium luteum purpurogenum,* does attack the carbohydrate portion of the meal.

2. Six-day-old cultures seem to hydrolyze the protein portion more in proportion to the carbohydrate part. Cultures 20 days old did produce more acid from the carbohydrate portion than from the protein. Cultures older than 20 days did not have any appreciable different effect.

3. The protein portion is hydrolyzed to a greater extent by this organism than by *Penicillium luteum purpurogenum* possibly to the peptone stage. Peptones are known to be a good source of nitrogen for *Aspergillus Niger* in the oxidation of carbohydrates, according to Bernhauer (1926), and Osburn and Werkman (1935).

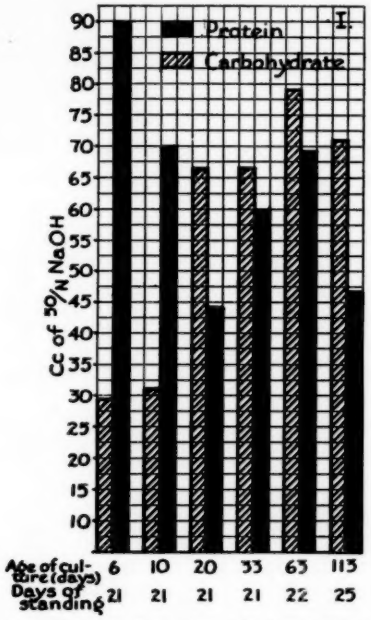

FIG. 1. Change in metabolic adaptation of *Aspergillus Niger* as determined by the Sorensen titration (Table 4). Cultures 20 days old, or older, show a less proteolytic effect.

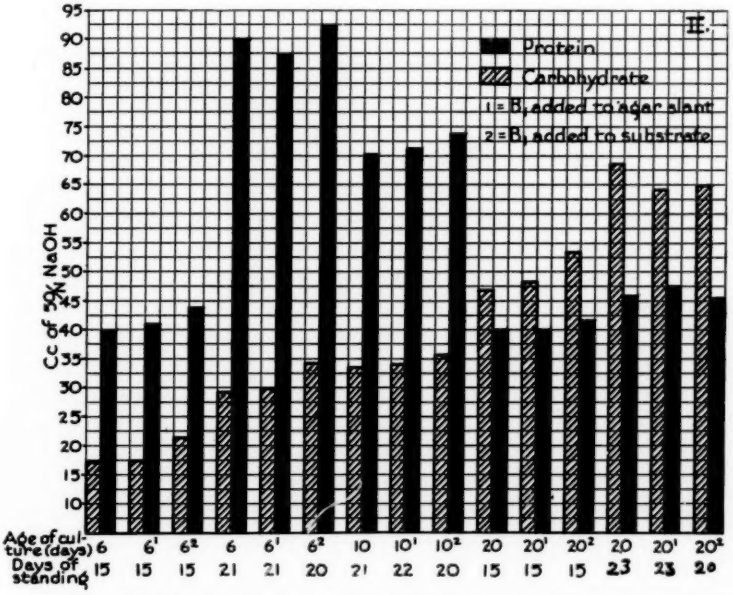
II.
Protein
Carbohydrate
1 = B1 added to agar plant
2 = B1 added to substrate
Cc of 30% NaOH
Age of culture (days) 6 6¹ 6² 6 6¹ 6² 10 10¹ 10² 20 20¹ 20² 20 20¹ 20²
Days of standing 15 15 15 21 21 20 21 22 20 15 15 15 23 23 20

FIG. 2. Metabolic adaptation change taking place in *Aspergillus Niger* as determined by the Sorensen titration (Tables 4, 5, and 6). The effect of vitamin B_1 can be seen here; while the presence of vitamin B_1 can be recognized as beneficial, it is not essential.

4. The growth-stimulating factor is beneficial but it must not be considered essential, Bowner (1937). There is no appreciable difference in the activity when vitamin B_1 is added to the agar so far as enzymatic action is concerned, Tatum *et al* (1934).

5. The metabolic adaptation taking place can be seen (Fig. 1 and 2). Cultures up to ten days of age show a more pronounced proteolytic activity in comparison with their carbohydrate fermentation. Using cultures 20 days old, or older, the activity of the enzymes with relation to the carbohydrates and proteins is reversed.

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CAROTENE CONTENT OF DIFFERENT VARIETIES OF GREEN AND MATURE SOYBEANS AND COWPEAS[1]

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The use of mature and green cowpeas and soybeans for human food as well as for livestock and poultry feed makes a quantitative study of the carotene content of different varieties of particular interest.

In spite of the large consumption of cowpeas, especially in the southern part of the United States, very little is known concerning their vitamin content. Only two reports on the vitamin A content of cowpeas were found in the literature. Jansen and Donath (1924) reported that cowpeas grown in the Dutch East Indies were a good source of vitamin A, ranking much higher than soybeans in this respect. Fraps and Treichler (1933) found that green air-dried cowpeas were low in vitamin A activity with only two Sherman-Munsell units per gram.

The vitamin content of soybeans, however, has received more attention. Miller and Robbins (1934) reported that green immature soybeans were a very good source of vitamin A. Reports in the literature on the vitamin A contained as carotene in mature soybeans, however, are very conflicting. Some investigators, Ivanova (1935) and Agnoli and Untersteiner (1936), have reported that mature soybeans were a very good source of vitamin A. Others, Daniels and Nichols (1917), Osborne and Mendel (1917), and Mitchell and Beadles (1936), gave them a fair rating; while Scheunert and Schlieblich (1935), Ligori (1934), and Jansen and Donath (1924) reported that mature soybeans were very low or completely lacking in vitamin A activity. Moreover, it has even been claimed by Frey, Schultz, and Light (1936) and Wilbur, Hilton, and Hague (1935) that soybeans exert an antagonistic action to vitamin A function.

In most of these studies the possible effect of varietal differences was disregarded. Since there are great differences in the nature and amount of pigmentation in the seed coats and cotyledons of different varieties of soybeans, it would seem that there might also be a wide range in their carotene content. Salmon (1927) has observed symp-

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toms of vitamin A deficiency in hogs receiving white corn and Tarheel Black soybeans, while a control lot receiving white corn and Mammoth Yellow soybeans did not show such symptoms.

In the present publication data are presented on the carotene content of a number of varieties of green and mature soybeans and cowpeas representing a wide range in seed pigmentation.

EXPERIMENTAL PROCEDURE

The different varieties of soybeans and cowpeas were grown on a sandy-loam soil fertilized with super-phosphate (400 pounds per acre) and muriate of potash (100 pounds per acre). Representative samples of green soybeans and cowpeas were picked when they were in their best stage for use as green vegetables, containing from 68 to 70 per cent moisture. They were brought to the laboratory where they were immediately shelled and analyzed. The remainder of the soybeans and cowpeas was allowed to stand in the field until mature, when they were harvested, shelled, and stored in covered galvanized cans for subsequent analysis. Excessive weathering of the mature seeds was prevented by picking each variety as soon as possible after the seeds had become dry and mature and before extensive shattering of the seed pods had occurred. Carotene analyses were made on all mature seeds within four months after they were harvested. Analyses made on a few varieties of soybeans stored for a year or more, however, showed that this precaution was unnecessary, since the carotene of soybeans stored under such conditions was apparently very stable.

The carotene content of the green soybeans and cowpeas was determined by a method somewhat similar in principle to the Peterson, Hughes, and Freeman (1937) modification of the Guilbert (1934) method for the determination of carotene in forage.

Grinding and Extraction of Fresh Green Soybeans and Cowpeas: A 10-gram sample of fresh green soybeans or cowpeas was thoroughly ground in a small glass mortar containing 15 ml. of a 10-per cent solution of KOH in methanol (synthetic). The material was transferred to an Erlenmeyer flask with 65 ml. of 10 per cent KOH in methanol and boiled for 45 minutes under a reflux condenser. After cooling, the supernatant alcohol solution was decanted into a 250-ml. separatory funnel. The residue was extracted twice more in similar manner with 40-ml. quantities of boiling methanol and once by boiling with 95 per cent ethyl alcohol (purified by distillation after standing several days over KOH) to facilitate subsequent extraction with skellysolve—skellysolve B, redistilled, boiling point 68 to 70°C.(154.4 to 158°F.). After the extraction with purified ethyl

alcohol, the residue was extracted by boiling with 40-ml. quantities of skellysolve until the extraction of pigment was complete (usually twice was sufficient).

Grinding and Extraction of Mature Soybeans and Cowpeas: The above methods of grinding and extraction were modified for the mature soybeans and cowpeas. Grinding was accomplished by means of an electric laboratory mill. Heating of the seeds during grinding was avoided by adding the seeds to the mill slowly, thereby preventing the accumulation of partially ground material in the mill. The material was sieved with a 20-mesh screen and the residue reground until it all passed through the screen. It was then mixed, and a 20-gram sample was weighed out and transferred to an Erlenmeyer flask for extraction. Although the alkaline-methanol extraction, which was always used with green soybeans and cowpeas, gave satisfactory results with the mature seeds, the bulkiness of the residue after the requisite 20-gram sample was subjected to alkaline-methanol extraction made centrifugation necessary for adequate separation of the extracts. This extra operation was eliminated by using skellysolve B instead of alkaline methanol in the extraction. The 20-gram sample of ground mature soybeans or cowpeas was boiled under a reflux condenser for 45 minutes with 80 ml. of skellysolve. After cooling and settling, the extract was decanted into a 250-ml. Erlenmeyer flask. The residue was extracted three more times by boiling with 40-ml. quantities of skellysolve and was then extracted with 40-ml. quantities of boiling methanol until the extracts were colorless; the residue was then discarded, usually after two such extractions. Solid KOH (eight grams) was added to the combined extracts, and the solution was evaporated on a boiling-water bath to about 150 ml. in volume. The solution was then transferred to a 250-ml. separatory funnel.

Separation and Purification of Carotene: Water was added to the combined extracts of the green or mature seeds contained in the separatory funnel in amount sufficient to reduce the alcohol concentration to about 85 per cent, thereby causing the skellysolve layer to separate. The funnel was gently shaken and the alcohol was drawn off into another 250-ml. separatory funnel and re-extracted with successive 10-ml. portions of skellysolve until the carotene was completely extracted. Since xanthophyll is appreciably soluble in skellysolve under these conditions, it continues to be extracted after the carotene extraction is complete; but by shaking a skellysolve extract with a small amount of 90-per cent methanol, the presence of carotene may be readily ascertained. The combined skellysolve extracts, which contained the carotene, xanthophyll, and a small amount of

chlorophyllines were extracted repeatedly in a 125-ml. separatory funnel with small portions of 90-per cent methanol until the chlorophyllines and xanthophyll were completely removed. These 90-per cent methanol extracts were combined and extracted once with a small amount of skellysolve to recover traces of carotene. The skellysolve solutions were combined and washed thoroughly with water. Emulsions, which usually formed when skellysolve solutions were washed with water, could be readily prevented by making the first two washings with a solution of Na_2SO_4 (about 100 grams per liter). The washed solution was transferred to a small Erlenmeyer flask and evaporated to 10 to 15 ml. volume on a water bath. The concentrated skellysolve solution was dried by adding one to two grams of anhydrous Na_2SO_4 and allowing it to stand for one-half hour. The solution was then transferred to a volumetric flask (usually 50 ml.) and made to volume with skellysolve.

Carotene Determination: The carotene content of the purified solution was determined with a universal spectrophotometer. The optical density readings were made at a wave length of 475 mμ. The carotene content of the solution was calculated from the equation $c = D/kl$, where c is the concentration of carotene in grams per liter of solution, D is the optical density as read from the spectrophotometer, l is the length in centimeters of the layer of solution, and k is the extinction coefficient for β carotene. The value 229, which was obtained for the extinction coefficient at 475 mμ of a skellysolve solution of carotene (S.M.A. β carotene recrystallized from petroleum ether—methanol 1:2) was used for k in all calculations. Results are expressed in γ of carotene per 100-gram sample on fresh and air-dry basis, respectively, for the green and mature materials.

Results from biological assays of soybeans and from chromatographic analyses of the purified extracts from several varieties of mature soybeans (to be published in detail) demonstrate that β carotene is the chief pigment in the solutions read on the spectrophotometer. If cryptoxanthin, which seriously complicates an accurate biological evaluation of "carotene" analyses of yellow corn, is present in soybeans, it exists only in such very small amounts that it can be disregarded in the conversion of carotene values to biological units.

EXPERIMENTAL RESULTS

The carotene content, expressed in γ per 100-gram sample, of 45 varieties of soybeans and nine varieties of cowpeas in the fresh green stage is given (Table 1). Most soybeans were found to be very good sources of carotene, but there was a wide range in the content of

TABLE 1

Carotene Content of Green Immature Soybean and Cowpea Varieties[1]

Soybean variety	Size	Carotene content
	No./10 gm.	*γ/100 gm. fresh*
Tanloxi	39	705
F.P.I. No. 93057	32	683
F.P.I. No. 84642	47	640
Biloxi	23	540
Matthews	33	509
Mamlox	28	497
F.P.I. No. 91425	41	497
F.P.I. No. 83868	....	480
F.P.I. No. 71543	44	476
Delnoshat	27	476
Ebony	42	474
Delsta	30	465
F.P.I. No. 85667	30	465
F.P.I. No. 85384	21	463
F.P.I. No. 85076	19	462
F.P.I. No. 82213	25	459
F.P.I. No. 94168	57	452
Mammoth yellow	29	452
Norredo	52	452
F.P.I. No. 94166	35	447
Mamotan	31	441
F.P.I. No. 84785	20	441
F.P.I. No. 19977	20	437
Easy-cook	33	430
F.P.I. No. 84734	27	430
F.P.I. No. 86022	24	430
George Washington	22	422
F.P.I. No. 96018	19	422
Peking	52	417
F. P. I. No. 85560	22	413
Chame	15	408
F.P.I. No. 95733	23	404
Hahto green selection	21	403
F.P.I. No. 86506	....	389
F.P.I. No. 85883	20	387
F.P.I. No. 64722	28	382
F.P.I. No. 87079	19	377
Hahto yellow selection	20	375
F.P.I. No. 83936	18	360
F.P.I. No. 85523	....	342
Rokusun	17	324
Kura	....	291
F.P.I. No. 60296	31	259
F.P.I. No. 82196	14	257
Higan	....	212
Cowpea variety		
Virginia black eye	46	231
Lady pea	....	230
Six-week pea	28	197
Brown crowder	....	179
Black crowder	....	179
White crowder	23	178
California black eye	....	175
Cream crowder	....	168
Dixie queen	....	140

[1] Moisture content 68 to 70 per cent.

different varieties of green soybeans; the high-carotene varieties contained about three times as much as did the low-carotene varieties. Fresh cowpeas were relatively low in carotene and showed a narrower variety range. Seven of the nine varieties of cowpeas analyzed contained less carotene than the lowest soybean variety and the other two were only slightly superior.

There was no apparent relationship between the size of the soybean and the carotene content. Although most of the low-carotene soybeans were large in size, a few of the high-carotene varieties were also large. Most of the small and medium-sized soybean varieties, however, were high in carotene. The carotene content of soybeans, as of other green plant materials, was closely correlated with the intensity of the green coloration. Several large varieties, which were pale green in color, were low in carotene. Nearly all of the small and medium-sized varieties, as well as a few of the larger ones, were

TABLE 2

Effectiveness of Skellysolve B and Alkaline-Methanol Methods of Extraction of Carotene From Mature Soybeans

Variety	Carotene content	
	Skellysolve B extraction (Authors' modification)	Alkaline-methanol extraction
	γ/100 gm.	*γ/100 gm.*
Peking	87.8	87.2
Matthews	118.0	117.3
F.P.I. No. 71543	80.3	81.9
George Washington	123.3	122.3

bright green in color and were high in carotene content. All of the varieties of immature cowpeas studied were pale green in color and were relatively low in carotene.

The analysis of mature soybeans and cowpeas was greatly facilitated by using skellysolve for the extraction of carotinoid pigments. That results obtained with this simplified method of extraction were in satisfactory agreement with those obtained with the alkaline- methanol treatment is shown (Table 2).

The results from carotene analyses of the 1936 and 1937 mature soybean and cowpea varieties, together with the seed coat and cotyledon color, are given (Table 3). Accurate evaluation of the intensity of yellow coloration in the cotyledons of the different varieties was impossible; but by placing cotyledons from each variety on small watch glasses and arranging them in order of color intensity, an approximate classification into bright, medium, and pale yellow cotyledons was effected.

TABLE 3

Carotene Content of Mature Soybean and Cowpea Varieties[1]

Soybean variety	Seed-coat color	Cotyledon color	Carotene content	
			1936 crop	1937 crop
			γ/100 gm.	*γ/100 gm.*
F.P.I. No. 93057	Bright green	Bright green	.....	243.5
F.P.I. No. 85076	Medium green	Medium green	210.0	200.0
F.P.I. No. 94168	Olive brown	Bright yellow	178.0	158.0
F.P.I. No. 94166	Olive brown	Bright yellow	197.0	151.0
Ebony	Black	Medium yellow	.....	136.0
F.P.I. No. 86022	Medium green	Medium green	172.3	.....
F.P.I. No. 86506	Medium yellow	Bright yellow	165.0	.....
F.P.I. No. 85384	Medium yellow	Bright yellow	162.5	112.0
George Washington	Olive brown	Bright yellow	.....	122.3
Matthews	Black	Bright yellow	.....	117.0
Norredo	Black	Medium yellow	.....	102.0
F.P.I. No. 82196	Mottled	Medium yellow	114.7	71.0
Hahto, green selection	Pale green	Medium yellow	100.2	72.6
Kura	Mottled	Bright yellow	.....	92.1
Peking	Black	Medium yellow	.....	87.4
F.P.I. No. 83868	Medium yellow	Bright yellow	82.0	83.5
F.P.I. No. 71543	Mottled	Bright yellow	.....	81.9
Mandell	Medium yellow	Medium yellow	81.6	.....
F.P.I. No. 84785	Mottled	Medium yellow	.....	79.2
Hahto, yellow selection	Medium yellow	Medium yellow	72.0	63.9
Chame	Brown	Medium yellow	.....	63.9
Easy-cook	Pale yellow	Medium yellow	41.9	61.7
F.P.I. No. 85523	Pale yellow	Medium yellow	65.5	59.0
F.P.I. No. 96018	Pale yellow	Medium yellow	76.3	55.6
Mammoth yellow	Pale yellow	Medium yellow	.....	50.2
F.P.I. No. 91425	Pale yellow	Medium yellow	25.6	53.0
F.P.I. No. 84642	Pale yellow	Medium yellow	67.4	45.4
Tokio	Pale green	Pale green	49.2	.....
F.P.I. No. 95733	Pale yellow	Medium yellow	44.6	49.1
Dunfield	Pale yellow	Medium yellow	40.0	.....
F.P.I. No. 85883	Pale yellow	Medium yellow	24.0	41.0
Tanloxi	Brown	Medium yellow	.....	39.4
Tarheel black	Black	Medium yellow	38.2	.....
Rokusun	Pale yellow	Pale yellow	29.1	38.2
Biloxi	Brown	Medium yellow	.....	37.2
Higan	Pale yellow	Pale yellow	.....	35.5
Mamredo	Pale yellow	Medium yellow	28.9	.....
Mamotan	Medium yellow	Medium yellow	25.6	.....
Delsta	Pale yellow	Medium yellow	17.5	24.7
Delnoshat	Pale yellow	Pale yellow	18.2	20.7
Mamloxi	Pale yellow	Pale yellow	22.9	19.1
Cowpea variety				
White crowder	White, brown eyes	White	20.8	32.8
Virginia black eye	White, black eyes	White	.....	27.3
Six-week pea	Light brown	White	.....	25.6
Brown crowder	Brown	White	.....	25.6
Lady pea	White	White	41.7	24.5
Cream-crowder	Brown	White	32.8	24.5
Dixie green	White, brown eyes	White	25.5	.....
Black crowder	Black	White	30.9	.....

[1] Moisture content 6 to 9 per cent.

A comparison of the results (Table 3 and Table 1) shows that there was considerable destruction of carotene during the maturing processes of all varieties of soybeans and cowpeas. If all results had been expressed on the moisture-free basis, this carotene destruction during maturing would appear about three times as great as is indicated by the values given in the tables. The amount of destruction in soybeans was found to vary greatly with varieties. In some varieties of soybeans and in all of the cowpea varieties a very large portion of the carotene was destroyed during maturation. Many varieties of soybeans, however, retained sufficient carotene in the mature seeds to be considered a fair source of vitamin A.

The bright green variety, F.P.I. No. 93057, was highest with 243 γ per 100 grams. Other varieties which retained a green color in the mature seed (F.P.I. No. 85076 and 86022) were also high in carotene. Three varieties—F.P.I. No. 94166, 94168, and George Washington—commonly used for hay, produced small, olive-brown-coated seeds which were relatively high in carotene. With one exception, Tarheel Black, a black coloration in the mature seed coat resulted in fairly high retention of carotene. The large, brown varieties, however, were consistently low. The four mottled varieties studied were intermediate in carotene content. The greatest variation within a color group was found in the mature soybeans having yellow seed coats and cotyledons. The intensity of the yellow pigmentation of the cotyledons proved to be only a fair index of carotene content. The four yellow soybeans highest in carotene had bright yellow cotyledons, whereas those with pale yellow cotyledons were low in carotene. There was a fairly wide range, however, in the carotene content of the medium yellow group, although there was no apparent difference in the intensity of their yellow pigmentation. This was undoubtedly due to the presence of large amounts of xanthophyll in soybeans which was responsible for a major portion of their yellow color.

Although the carotene content of the mature seeds from those varieties of soybeans and cowpeas which were grown during two successive years (1936 and 1937) were not in absolute agreement, the results came within the same general range. These seasonal variations within the same variety are apparently due to non-genetic factors.

The length of time the soybeans were allowed to stand in the field after they had reached the dry, mature stage had a marked effect upon their carotene content. The effect of excessive field ripening is shown (Table 4). In this study a portion of the rows of 11 varieties of soybeans was allowed to stand in the field for a period

of from two to three weeks after the main portion of the mature seeds had been harvested. They were then picked and stored as usual in galvanized cans at room temperature for subsequent analysis. In all of the varieties tested, excessive field ripening caused a marked destruction of carotene which in some instances exceeded 50 per cent in two weeks. Since there was no significant loss of carotene from mature soybeans stored in dark containers in the laboratory, it is probable that the destruction in the field was caused by sunlight and may have been influenced by moisture resulting from heavy dews. Although the mature soybeans were retained in the seed pods during this period of extended field ripening, the seed

TABLE 4

Effect of Extended Field Ripening Upon Carotene Content of Mature Soybeans[1]

Variety	Carotene content	
	Early harvest	Late harvest
	γ/100 gm.	*γ/100 gm.*
F.P.I. No. 84734	90.0	32.8
F.P.I. No. 85667	54.0	34.4
F.P.I. No. 60296	25.6	23.4
F.P.I. No. 82213	70.0	38.3
F.P.I. No. 64722	35.5	23.4
F.P.I. No. 84166	162.0	61.8
F.P.I. No. 93057	243.0	173.0
F.P.I. No. 87079	101.0	69.3
Mammoth yellow	50.2	38.2
Easy-cook	61.7	33.9
Mamlox	19.1	12.0

[1] Moisture content 6 to 9 per cent.

pods of most varieties had become so translucent that they afforded little protection to the seed. The seed pods of one variety, F.P.I. No. 93057, developed a black pigmentation during the late stages of maturation. It is interesting to note that this variety retained a larger amount of carotene during maturation than any other variety.

SUMMARY

Results are reported on the carotene content of different varieties of green and mature soybeans and cowpeas. Most varieties of soybeans in the green stage were found to be very good sources of carotene with a variety range from 212 to 705 γ per 100-gram fresh sample. The varieties of cowpeas studied contained from 140 to 231 γ per 100-gram fresh sample. The carotene content of different varieties of mature soybeans ranged from 18 to 243 γ per 100-gram air-dry sample. The relationship of seed-coat and cotyledon color to

carotene content is discussed. All varieties of mature cowpeas analyzed were low in carotene content, being comparable to the lowest soybean varieties. Excessive weathering of soybeans, produced by allowing plants to stand in the field two to three weeks after the seeds reached maturity, caused a destruction of as much as 50 per cent of the carotene in several varieties of mature soybeans.

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A SUMMARY OF STUDIES ON POLLUTION IN SHELLFISH [1]

ESCHERICHIA COLI VERSUS THE COLIFORM GROUP AS AN INDEX OF FECAL POLLUTION AND THE VALUE OF A MODIFIED EIJKMAN TEST

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In any discussion of the use of coliform bacteria as an index of pollution, it should be borne in mind that the present "Standard Methods of Water Analysis" of the American Public Health Association have been developed for the examination of water and particularly for treated waters. A method for determining the fitness of (treated) drinking waters for human consumption, obviously, may not be suitable for determining probable fecal pollution in shellfish or the waters in which they grow. There is a tremendous difference in the two situations and the purpose of the tests.

In the bacteriological examination of drinking waters and particularly of treated waters for drinking purposes it is practical, in most instances, to filter, chlorinate, or protect the water to the exclusion of practically all bacteria of the coliform group. Because it is so easy to exclude all coliform types of bacteria and because of the long and rather futile effort to decide which of these bacteria are fecal and which not, the only practical course, so far as drinking water is concerned, is to aim at the exclusion of all coliform bacteria. While we can all agree in this, a wise course would seem to be to modify the present methods of water analysis to provide a much simpler test for bacteria of the coliform group and add a test for *Escherichia coli*. There are many untreated drinking waters subject to certain types of contamination which are apparently of little importance from the standpoint of fecal pollution. It has been found of value to be able to determine the numbers of both coliform bacteria and *Escherichia coli* in such supplies. It is customary for sanitary engineers to investigate any water supply with excessive numbers of coliform bacteria. Frequently no evidence of fecal pollution can be found and the innocuous character of such contamination is supported by the total absence of *Escherichia coli*.

FECAL POLLUTION IS GREATEST PROBLEM

It is not practical to so treat all shellfish-growing waters nor is it practical or necessary to exclude all types of coliform bacteria

[1] Revision of talk given at a meeting of the Maryland Branch, Society of American Bacteriologists, Baltimore, Maryland, May 10, 1938.

from shellfish themselves. Large numbers of coliform organisms may be present in oysters and other shellfish that are not subject to fecal pollution of any consequence. This condition is largely a factor of temperature (Fig. 1 and 2). Consequently, those who have been concerned with the sanitary control of shellfish in more northern waters, have found the coli-aerogenes group reasonably satisfactory as an index of pollution for shell stock. This is probably due to the predominance of *Escherichia coli* in colder waters and the relative scarcity of other coliform bacteria. Those who have been concerned with this problem in more southerly waters have, on the other hand, experienced so much difficulty with high scores (number of bacteria

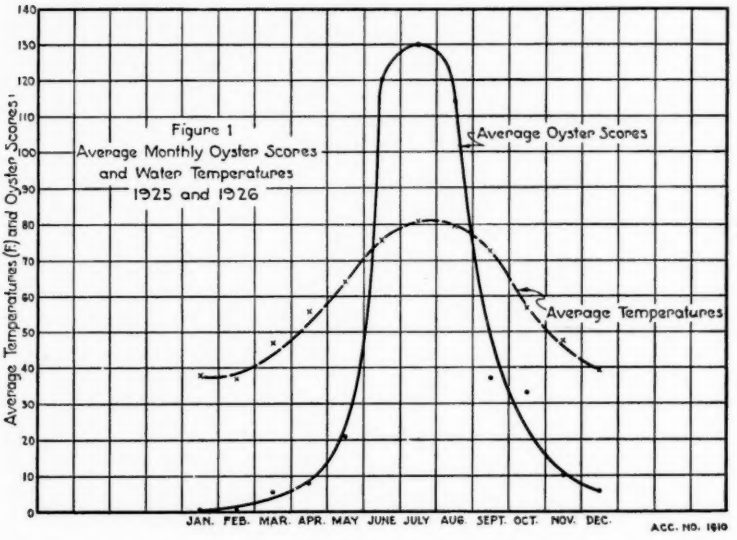
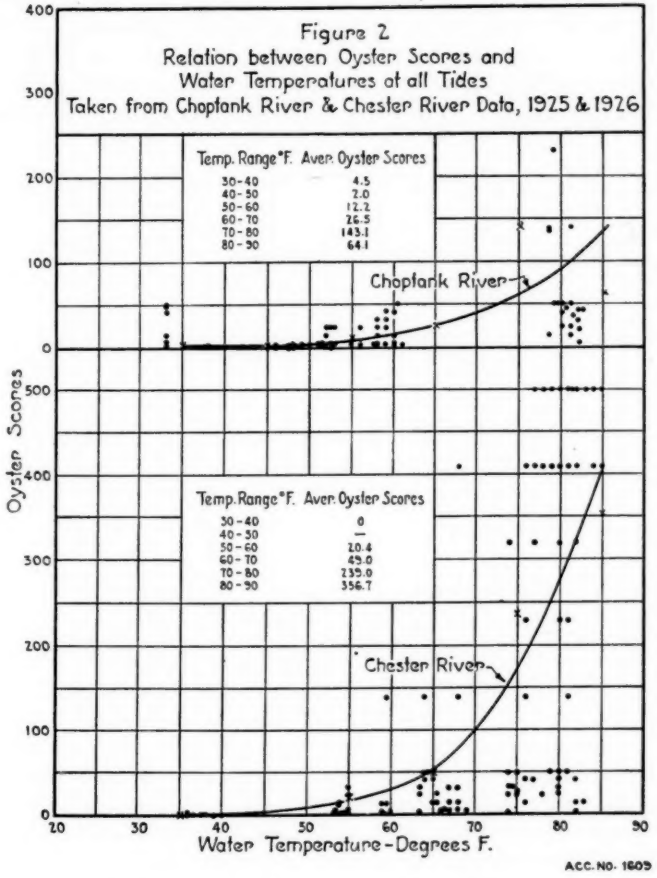


Figure 1
Average Monthly Oyster Scores
and Water Temperatures
1925 and 1926
Average Oyster Scores
Average Temperatures
Average Temperatures (F.) and Oyster Scores
JAN. FEB. MAR. APR. MAY JUNE JULY AUG. SEPT. OCT. NOV. DEC.
ACC. NO. 1610

of the coli-aerogenes group in five c.c.), that they have been forced to ignore such determinations and to depend on the number of coliform organisms in the waters over oyster beds. The use of the coli-aerogenes group as an index of fecal pollution in shucked oysters has given almost universal trouble, and the tendency now is to ignore such findings on the basis that they do not indicate fecal contamination. Unfortunately the problem remains and cannot be solved by ignoring an unsatisfactory method. Some better means of determining probable fecal pollution in shellfish is urgently needed. In short, many of those who have studied this problem and have had to use the present method have long been convinced that high scores based on coli-aerogenes bacteria frequently have no value as indicators of fecal pollution.

ESCHERICHIA COLI MOST CHARACTERISTIC

There is, of course, no question in anyone's mind that *Escherichia coli* is the most characteristic and constant coliform bacterium of the human intestine and of fresh human feces. We know that less frequently *Aerobacter aerogenes* may also be found in large numbers and that at times it may become the predominant and rarely the



only type. The *Citrobacter* types are not found to any extent in human feces except possibly in the feces of rice-eating Orientals. We know that *Aerobacter cloacae* is difficult to find at all times. The *cloacae* strains found in oysters seem to differ somewhat from those found in human feces, according to Perry (1930).

In unpolluted oysters large numbers of coliform bacteria may be found when the temperature of the growing waters is relatively high—above 15.6°C.(60°F.)—or oysters are subjected to relatively high temperatures during handling. H. D. Pease (personal communication) has found that oysters exposed to the heat of the sun on the decks of dredging boats may develop very high scores. High scores are frequently found in freshly shucked oysters (Table 1) or in those on the market (Table 2). What then is the explanation for such high coliform scores? Many examinations have revealed that such high scores are usually due to the presence in oysters of *Aerobacter cloacae*, and when there is little or no fecal pollution there are few, if any,

TABLE 1

Escherichia Coli and Coliform Group Bacteria[1] *in Freshly Shucked Oysters*

Laboratory No.	*Escherichia coli*	Coliform group
76	0	16,000
80	0	1,400
81	0	1,300
1	0	18,000+
13	350	1,100
41	1,700	18,000+
70	0	18,000+
74A	0	1,400
77B	20	1,200
80	0	9,000
97	20	2,500

[1] Relative numbers per 100 grams.

Escherichia coli present. For example, in the case of high scores in oysters from the Chester River, Maryland, no *Escherichia coli* could be found as the locations from which the oysters were taken were far from the sources of fecal pollution. High scores are frequently obtained on oysters from shucking houses where there is no evidence of gross mishandling. If the types of coliform bacteria are determined, they are often found to be mostly *Aerobacter cloacae*.

An attempt has been made to correlate the coliform and *Escherichia coli* scores in oysters with probable pollution (Table 3). Little difference has been found in the numbers of coliform bacteria in oysters whether they have been subjected to no known fecal pollution or to moderate or heavy fecal pollution, while in the case of *Escherichia coli* there is a distinct correlation.

In the case of the oyster-growing waters (Table 3) there is a considerable correlation between coliform bacteria and estimated

fecal pollution. This is due, no doubt, to the growth of certain coliform types in polluted streams as has been cited by Hamilton (1935) in his report to Pearson on studies of the Whangpoo river in China. Hamilton correctly interprets these results, it is believed, when he claims that the *Aerobacter* types indicate the process of self-purification rather than fecal pollution. This interpretation is in accord with the author's observations, over a number of years, on stream pollution in connection with the pollution of shellfish and is, he believes, the correct interpretation of the relative numbers of coliform bacteria found in oyster waters (Table 3). The extent of fecal pollution is more appropriately indicated by *Escherichia coli* and the correlation is better.

Some idea of the relative scarcity of *Escherichia coli* and the relatively large numbers of other coliform types found in freshly

TABLE 2

Escherichia Coli and Coliform Group Bacteria[1] *in Market Oysters—December, 1935*

Laboratory No.	*Escherichia coli*	Coliform group
689	20	80
690	0	18,000+
691	0	80
692	0	18,000+
693	20	500
694	0	1,800
695	110	200

[1] Relative numbers per 100 grams.

shucked oysters is given (Table 1). One can hardly imagine, if these coliform types came from the fecal matter on the hands of shuckers, that any such picture could possibly exist. Such findings simply do not represent fecal pollution and to so interpret coliform scores is totally unwarranted.

A recent report by Dodgson (1937), late director of the Ministry of Agriculture and Shellfisheries of Great Britain, has added another bit of evidence of the most valuable and dramatic type. He found that many coliform bacteria in shellfish have no significance from the standpoint of fecal pollution and that an attempt to so use them can result only in confusion or absurdity. Over 12,000 tons of mussels have been "conditioned" by chlorination of the water in which they are purified at Conway, England, since a plant was started there in 1916. This plant, probably the finest in the world, operated very successfully, so far as the reduction of mussel

scores was concerned, until the years 1935 and 1936 when, like a bomb, mussels from this plant were found on the market with enormous numbers of coliform bacteria. After great anxiety and study, all these coliform bacteria were found to be of the *cloacae* type. Further study revealed that conditions, for some unknown reason, had been favorable for the proliferation of this type in the mussels. Examination of barnacles revealed this same *cloacae* type present and growing in enormous numbers. No *Escherichia coli* were found and Dodgson states that everyone who studied the matter was satisfied that this *cloacae* type had no fecal significance. He cites the recovery of a similar coliform organism by the millions from the intestines of gnats and also from other sources. This experience furnishes clear-cut and convincing evidence of a coliform bacterium of no fecal connection, occurring in enormous numbers through multiplication

TABLE 3

Relationship of Escherichia Coli and the Coliform Group to Fecal Pollution in Oysters and Oyster Waters in Various Tributaries of Chesapeake Bay

Fecal pollution as judged by sanitary survey	Pollution as indicated bacteriologically [1]			
	Oysters		Oyster waters	
	Esch. coli	Coliform group	*Esch. coli*	Coliform group
Not significant	4.5(422)[2]	1,158(694)	1.5(238)	61(1,026)
Slight	49.0(230)	1,979(476)	5.5(287)	100(1,752)
Moderate	196.0(97)	1,634(184)	36.0(92)	134(686)
Heavy	167.0(30)	1,509(97)	67.0(67)	376(413)

[1] Most probable numbers per 100 c.c.—McCrady. [2] Figures in parentheses represent number of examinations—*Esch. coli* determinations: oysters 779, water 684, total 1,463; coliform group determinations: oysters 1,451, water 3,877, total 5,328.

(Dodgson calls them "multipliers"), causing unfounded anxiety and yet lacking apparently any significance from the standpoint of fecal pollution. Probably no more forceful illustration of the need of excluding questionable coliform bacteria in shellfish examination for evidence of fecal pollution could be secured.

In the examination of shellfish for evidence of fecal pollution the use of the coliform group of bacteria has been found impractical and unsatisfactory under many conditions. Evidence indicates that a procedure based on the sole use of *Escherichia coli* would eliminate many of the difficulties owing to confusing non-fecal coliform bacteria with those of probable fecal origin.

In a still more recent publication Dodgson (1938) states, "There can be no legitimate standard of purity for purified shellfish based on tests carried out at a temperature of 37°C." He goes on to ask, "What then is the solution?" and answers, "My own view is that

a perfectly satisfactory one (test) is to be found in incubators at 44°C. For the last two years we have carried out a series of experiments at 40°, 42°, 44° and 45°C. with controls at 37°C. I cannot, of course, enter into any analysis of results now; suffice to say that at 44°C. all the citrate-positives derived from shellfish—that is to say the 'multipliers' responsible for anomalous results—are eliminated while the great majority of typical *B. coli* grow. I would depreciate any 'hair-splitting' about the significance of borderland bacteria and what not. Precise knowledge is lacking (in regard to fecal and non-fecal types) and if we do not confine ourselves to broad issues, we shall get nowhere." These observations are, as will be evident from this paper, in complete agreement with those of the author.

PREVIOUS WORK ON PRACTICAL ISOLATION TEST

With this background the need for a specific test for *Escherichia coli* is obvious. If a test for coliform bacteria in general is needed, this is provided by primary incubation at 37°C.(98.6°F.) with confirmation in a secondary liquid medium, such as brilliant green, as provided for in the eighth edition of "Standard Methods of Water Analysis." Such a relatively short method for coliform bacteria would save time and expense which could well be put into tests for *Escherichia coli*. One cannot but conclude from knowledge of the lack of fecal significance of certain coliform types in shellfish and shellfish-growing waters, that a certain amount of confusion in the interpretation of coliform estimates for streams and untreated drinking water results from the use of a crude coliform index. Fortunately the situation for drinking waters, as has been pointed out, is such that the crude index can be practically applied. Since there is, however, a real need for a more practical method for the isolation of *Escherichia coli* from shellfish and shellfish waters and even certain drinking waters, the work of Perry and Hajna (1933), in an effort to improve the Eijkman test for this purpose, is reviewed briefly here. It is recognized, of course, that refinements can still be made. This is true of practically all accepted tests. A modified Eijkman test, however, has been practically applied in Maryland for a number of years.

Interest in the Eijkman test dates back ten years to the time of trying to determine the significance of various coliform bacteria in oysters, Perry (1928, 1929). A better test than lactose broth was needed, that is, one which would be selective for *Escherichia coli*. While a number of investigators had used the test proposed by Eijkman in 1904 with both satisfactory and unsatisfactory results,

the principle of the temperature selection seemed sound. Leiter (1929) studied this test and found it very satisfactory. Perry decided, therefore, that the test should be tried out on the cultures isolated from oysters and oyster waters. Every one of his cultures which had been identified as *Escherichia coli* produced gas in Eijkman's medium at 46°C.(114.8°F.), while *Aerobacter* strains almost invariably failed to give gas in this test. He concluded, therefore, that selective growth of *Escherichia coli* from other members of the coliform group could be effected by incubation at 46°C. Furthermore, incubation at 46°C. offered a positive presumptive test

TABLE 4

Comparative Results, Both Presumptive and Confirmed, for Escherichia Coli and Coliform Organisms in Raw Sewage With Various Media and With Eijkman Broth[1]

	Standard lactose	Standard lactose plus K_2HPO_4	Eijkman	Ritter's	Salle's	Dom-onick-Lauter's	Stark and England's
Total tubes inoculated..	270	270	270	270	270	270	270
Presumptive gas tubes..	185	181	174	177	171	178	183
Confirmations, coliform group.....	182	180	171	176	171	178	183
Confirmations, *Esch. coli*.....	153	105	161	141	80	155	148
Per cent of tubes showing gas.....	68.5	67.0	63.3	65.5	63.3	65.9	67.8
Per cent of inoculated tubes confirmed for *Esch. coli*.....	56.7	39.0	59.6	52.2	29.6	57.4	54.9
Per cent of gas tubes confirmed for *Esch. coli*	82.8	58.0	92.5	79.8	46.8	87.0	81.0

[1] Ten samples examined.

for *Escherichia coli*. All other tests were of value primarily only for pure cultures and did not preclude the necessity of primary cultivation in lactose broth with frequent overgrowth by other coliform and non-coliform bacteria with loss of *Escherichia coli*. Confirmation of gas tubes was relatively easy, as streaking from these on eosin-methylene-blue plates usually resulted in almost pure cultures of *Escherichia coli* in contrast with the mixture of spreading mucoid colonies of *Aerobacter* and other types from 37°C.(98.6°F.) lactose-broth tubes which make the isolation of *Escherichia coli* so difficult. Further difficulty in confirmation of *Escherichia coli* on eosin-methylene-blue plates from 37°C. lactose-broth tubes, results

from inability to distinguish *Citrobacter* colonies from those of *Escherichia coli*. The Eijkman test inhibits *Citrobacter* types and prevents much of this difficulty.

One of the unsatisfactory conditions in the use of the original Eijkman test was the dying off of the *Escherichia coli* in gas tubes within 48 hours and at times within 24 hours. This was a distinct practical handicap. The remedy seemed simple. The original Eijkman medium contained a large amount of glucose which resulted in the rapid formation of a lethal amount of acid. This was controlled by reducing the sugar to 0.3 per cent and by the addition of phosphates to neutralize the acid from this amount of glucose. Meat extract was found to reduce gas production and peptone was substituted. The formula was a simple one, yet so far Perry and Hajna

TABLE 5

Comparative Results, Both Presumptive and Confirmed, for Escherichia Coli and Coliform Organisms in Eijkman Medium and Standard Lactose Broth, From Oysters, Oyster Waters, and Crabmeat

Sample	Media	Inoculated tubes	Presumptive gas 24 and 48 hours	Confirmations		
				Coliform group	*Escherichia coli*	
					Number	Per cent
Oysters (shucked)	S.L.B.[2]	2,195	1,068	890	86	8.1
182	Eijk.	2,195	422	366	204	48.3
Water (oyster-growing) 344	S.L.B.	3,239	853	637	284	33.3
	Eijk.	3,233	406	352	213	51.9
Crabmeat	S.L.B.	891	288	243	50	17.4
88-229[1]	Eijk.	2,170	425	363	252	59.2
Crabmeat	S.L.B.	801	237	196	37	15.6
78	Eijk.	801	126	105	80	63.5

[1] Eighty-eight samples examined in lactose broth at 37°C.(98.6°F.), 229 in Eijkman broth. [2] S.L.B. = standard lactose broth

have not been able to improve on it except for the use of lactose which will be referred to shortly.[2]

This medium was tested (using dextrose) with pure cultures of coliform bacteria and with coliform bacteria in feces, by decimal dilutions of specimens, Perry and Hajna (1933). In this way they determined the highest dilutions of human feces yielding *Escherichia coli* in their modified medium as compared with lactose. An average of 41 per cent more *Escherichia coli* was obtained on 24 specimens with the modified Eijkman medium than with standard lactose broth.

[2] Formula for modified Eijkman test: bacto peptone 1.5 per cent; bacto lactose .3 per cent; K_2HPO_4 .4 per cent; KH_2PO_4 .15 per cent; NaCl .5 per cent.

In another study (Table 4) the modified Eijkman medium was compared with (a) standard lactose broth, (b) buffered standard lactose broth, (c) Ritter's basic fuchsin lactose broth, (d) Salle's crystal violet lactose broth, (e) Dominick-Lauter's brom-cresol-purple methylene-blue lactose broth and Stark and England's formate ricinioleate broth, on samples of raw sewage, Hajna and Perry (1935). The modified Eijkman medium was found to yield more *Escherichia coli* and less false presumptives than any of the other media.

In a fourth paper (Table 5) are presented results for *Escherichia coli* obtained by using a modified Eijkman medium and standard

TABLE 6

Comparative Numbers of Escherichia Coli Isolated From Oysters and Oyster-Bearing Waters, Using Parallel Inoculations in Lactose Broth and Modified Eijkman Broth as Primary Media

Material	Samples	*Escherichia coli*[1]		Coliform group[1]	Year
		Lactose	Eijkman	Lactose	
Shucked oysters	182	86.0[2]	204.0[2]	890.0[2]	1934
Shucked oysters	17	7.7	9.0	3,545.0	1935
Shucked oysters	51	9.3	41.0	1,488.0	1936
Shell oysters	156	10.2	23.8	725.0	1935
Shell oysters	44	33.8	53.7	502.0	1936
Oyster waters	324	9.1	17.7	30.3	1935
Oyster waters	186	9.1	9.3	20.2	1936

[1] Most probable numbers per 100 c.c. [2] Number of tubes confirmed.

lactose broth on a total of 833 samples of shucked oysters, oyster waters, and crabmeat involving 15,525 primary fermentation tube inoculations, Perry and Hajna (1935). They obtained 137 per cent more confirmations for *Escherichia coli* by the Eijkman method with shucked oysters, 25 per cent less for Eijkman broth with the water samples, and 109 per cent more by the Eijkman method for crabmeat.

A summary of the respective numbers of *Escherichia coli* isolated in parallel inoculations from shell and shucked oysters and oyster-growing waters for several years, is given (Table 6). In spite of the fact that the method for isolation of *Escherichia coli* has been improved considerably since 1936, on the whole, far more *Escherichia coli* have been consistently isolated from oysters and oyster waters at 46°C.(114.8°F.) than at 37°C.(98.6°F.).

Hajna (1937) subsequently studied the decomposition of various carbohydrates (monosaccharides: xylose, arabinose, glucose, mannose, and fructose; disaccharides: trehalose, sucrose, maltose, and lactose; and alcohols: mannitol, sorbitol, dulcitol, and adonitol) at

46°C. He found all the substances tested to be fermented at 46°C. as readily as at 37°C. if a suitable basic medium was used, with the exception of dulcitol and adonitol. He also observed that buffers enhanced gas production both at 37°C. and 46°C., while meat extract restrained it.

Subsequent to this, a sixth study by Hajna and Perry (1938) was made of the practicability of substituting lactose for glucose in the modified Eijkman medium and the value of adding sodium formate to both the dextrose and lactose media. By the substitution of lactose for glucose in the original formula, the number of isolations of *Escherichia coli* from 25 sewage samples was markedly increased. Sodium formate was used in the glucose formula and was found to increase the yield of *Escherichia coli* though somewhat more false presumptives were obtained. The addition of sodium formate to the

TABLE 7

Isolation of Escherichia Coli at 46°C.(114.8°F.) From Tubes of Dextrose and Lactose Media Similarly Inoculated With 25 Samples of Sewage

	Media				
	S.L.[1]	D46[2]	L46[3]	DF46[4]	LF46[5]
Total tubes inoculated[6]	585	585	585	585	585
Tubes having gas at 48 hours	330	231	265	269	259
Total confirmations, coliform group	306	210	245	243	231
Total confirmations, *Escherichia coli*	178	184	230	200	213
Per cent of gas tubes confirmed for coliform group	92.8	90.9	92.5	90.4	89.2
Per cent of gas tubes confirmed for *Escherichia coli*	54.0	79.7	86.7	74.4	82.2

[1] S.L.=standard lactose broth, 8th ed., "Standard Methods of Water Analysis," A.P.H.A. [2] D46=basic medium plus dextrose at 46°C. [3] L46=basic medium plus lactose at 46°C. [4] DF46=same as D46 with addition of .1 per cent of sodium formate. [5] LF46=same as L46 with addition of .1 per cent of sodium formate. [6] Each dilution inoculated in triplicate up to $10^{-2.0}$.

lactose medium both decreased the yield of *Escherichia coli* and increased the number of false presumptives.

Lactose has also been found superior to dextrose in the medium for the isolation of *Escherichia coli* from oyster-growing waters, according to Perry and Hajna (1939), (Table 8).

Dodgson (1938) has reported the successful use of MacConkey's medium at temperatures of 42 and even 44°C.(107.6 and 111.2°F.). Studies on the optimum temperature have shown that a tube temperature of 45.5°C.(113.9°F.) is close to the optimum, Hajna and Perry (1939), (Table 9). *Citrobacter* types fail to grow above 45°C. (113°F.) and most *Aerobacter* types at even lower temperatures. Studies on incubator temperatures are not yet completed but with a

large series of cultures of the genera *Escherichia, Aerobacter,* and *Citrobacter* at various temperatures both in an incubator and in a water bath, practically all of the *Escherichia coli* strains grew at 46°C.; while the *Aerobacter* and *Citrobacter* strains almost completely inhibited. While lower temperatures do not offer the same degree of selective action for *Escherichia coli,* it is possible that a temperature of 45 or even 44°C. may prove more practical for general laboratory use. While selective action for *Escherichia coli* has not been found as satisfactory at these lower temperatures, most of the *cloacae* strains which Dodgson refers to as "multipliers" and which are the most frequently encountered coliform types in shell-

TABLE 8

Comparison of Dextrose and Lactose Media at 46°C.(114.8°F.) With Standard Lactose Broth at 37°C.(98.6F.) When Inoculated With Oyster-Growing Waters

Media	Number of samples	Inoculated tubes	Gas tubes	*Esch. coli*
Stand. lact. broth 37°C.[1]		120	45	35
vs. stand. lact. broth 37°C.	8	120	44	34
Stand. lact. broth 37°C.		120	49	31
vs. dextrose broth 46°C.	8	120	15	13
Stand. lact. broth 37°C.		1,350	554	322
vs. lactose broth 46°C.	54	1,350	499	467
Stand. lact. broth 37°C.	70	1,750	718	378(546[2])

[1] Control, using parallel inoculations. [2] Secondary confirmation for *Escherichia coli* by transferring from primary 37°C. lactose-broth gas tubes to lactose broth at 46°C.

fish, are inhibited at these temperatures. A temperature of 45° ± .5°C would sacrifice some selection but would avoid the risk of incubators exceeding 46°C. with inhibition of *Escherichia coli.* The test requires a good water-jacketed incubator and a sensitive and dependable thermo-regulator. For optimum results, the final concentration of medium when mixed with the water under test, should be constant.

Past experience indicates that an incubator is preferable to a water bath. MacConkey's medium has been found by Hajna and Perry (1939) to be less selective than their modified Eijkman medium whether used in an incubator or a water bath. There is a lag of an hour or two before the tubes reach the temperature of the incubator. This slight incubation at a lower temperature seems valuable; it has been used successfully by the author for ten years. The greatest value of the Eijkman test is on samples having a relatively

high content of *Aerobacter* types. These are apt to overgrow any *Escherichia coli* in lactose-broth tubes at 37°C.(98.6°F.), or their colonies are apt to overgrow the colonies of *Escherichia coli* on eosin-methylene-blue plates. Higher temperatures, however, cannot be used with the same disregard of close temperature control as is possible with lactose broth at 37°C.

There is an increasing need for a practical method for the isolation of *Escherichia coli*, which bacterium, we feel certain, is bound to receive eventually the recognition by sanitarians which is justified by its position of preëminence in the coliform group as an indicator

TABLE 9

Gas Production at 42, 44, and 46°C.(107.6, 111.2, and 114.8°F.) by Coliform Bacteria

Coliform type	No. of strains	42°C.			44°C.			46°C.		
		D	L	M	D	L	M	D	L	M
Aerogenes....	120	106	103	107	77	88	102	3	5	13
Cloacae.....	60	14	26	39	5	4	10	0	0	1
Citrobacter..	45	39	16	37	22	5	29	0	0	1
Escherichia.	1,374	....	....	....	....	....	....	1.370	1,369	1,374

Reactions[2] *of Cultures*

	MR	VP	I	Gly.	Gel.	Cit.	Ur.	Hip.
Aerogenes.....	—	+	—	+	—	+	+	+
Cloacae.....	—	+	—	—	+	+	+	—
Citrobacter.....	+	—	—42, +3	+42, —3	—	+	—	—
Escherichia.....	+	—	—30, +1344	—	—	—	—	—

[1] Sources of strains are as follows: *Aerogenes*—human feces 29, sewage 24, water 23, crabmeat 10, oysters 24, grain 5, soil 5; *Cloacae*—human feces 23, sewage 15, drinking water 12, crabmeat 2, oysters 8; *Citrobacter*—various; *Escherichia*—human feces 395, animal feces 134, crabmeat 16, oysters 68, oyster waters 112, drinking waters 196, swimming pool waters 23, raw sewage 430.

[2] MR=methyl red, VP=Voges-Proskauer, I=Indole, Gly.=glycerol fermentation, Gel. =gelatin liquefaction, Cit.=citrate growth, Ur.=growth in uric acid medium, Hip.=hippurate hydrolysis.

of fecal pollution and which the keen mind of that master bacteriologist, Theobald Smith, recognized years ago when he said, "Arguments for or against the significance of the colon group in water can only be properly dealt with when all shall have agreed upon what is to be called *Bacillus coli communis (Escherichia coli)* and when related forms shall have been assigned to their proper places."

SUMMARY

While the coliform group is a very satisfactory test for determining the freedom of (treated) drinking water from the ordinary enteric pathogens, great value would result from the use of a relatively simple test for coliform bacteria and an additional test for *Escherichia coli*.

The examination of shellfish and their growing waters cannot be considered in the same category with drinking water which can be filtered, chlorinated, or protected. Many coliform bacteria, particularly of the *cloacae* types, are present in shucked market oysters or shell oysters when the temperature exceeds 15.6°C.(60°F.) but are without significance as indicating pollution. *Escherichia coli* would seem to be the logical bacterium to use as an index of fecal pollution in shellfish and shellfish-growing waters.

The medium proposed by Eijkman in 1904 for the selective growth of *Escherichia coli* at 46°C.(114.8°F.) from natural mixtures with other coliform bacteria in water, oysters, etc., has been modified and used successfully and routinely for a number of years in the bacteriological laboratories of the Maryland State Department of Health. Recently lactose has been found better than dextrose when substituted in a modified Eijkman medium for the isolation of *Escherichia coli.*

ACKNOWLEDGMENT

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NATURE OF A YELLOW DEPOSIT IN JARS OF HOME-CANNED GREEN ASPARAGUS[1]

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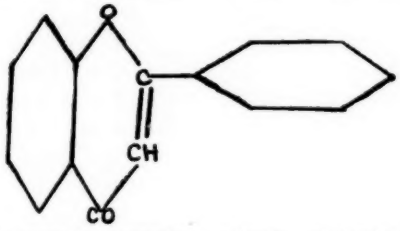
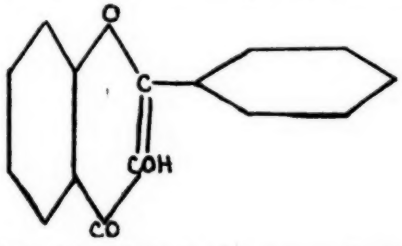
A yellow deposit has been observed frequently in glass jars of home-canned green asparagus. A search of the literature indicates that its nature and possible causes have apparently not been previously discussed, although it not infrequently happens that jars of asparagus showing such deposits are regarded with suspicion and discarded.

Pending determination of the factors responsible for the occurrence of this yellow sediment, some investigations of its possible nature have been made, the results of which are given here briefly.

According to Bernthsen (1922) and others, the parent substance of a number of yellow pigments which occur in the vegetable kingdom is flavone, which is regarded as being a benz-2 phenyl-y-pyrone.



The flavonols may be regarded as hydroxyl derivatives of flavone.



Onslow (1923) states that the flavones or flavonols are usually present as glucosides in plants, one or more of the hydroxyl groups being replaced by some sugar. The sugar residue may be either glu-

[1] Food Research Division Contribution No. 405.

cose, rhamnose, or some other monosaccharide or even a disaccharide. The methylpentoses are common in this latter group, according to Armstrong (1919).

Both Onslow and Armstrong also state that on hydrolysis of such glucosides with dilute acids, the sugar is split off and that at the same time the color deepens and a deposit of the pure pigment is formed, since the latter is less soluble than the glucoside.

The sediment in jars of canned green asparagus has been noted to consist of aggregates of fine, needle-like crystals. These are soluble in hot water and alcohol but insoluble in ether. This observation agrees with the statement of Onslow regarding the solubility of glucosides of the flavone series.

When the crystals are dissolved in water, they impart a very pale yellow color to the aqueous solution. On the addition of concentrated HCl, 10 per cent NaOH, or NH_4OH to the solution, the yellow color is strongly intensified. An olive-green color is obtained with ferric chloride solution, and a yellow precipitate occurs with basic lead acetate solution. An orange color is obtained when uranium acetate solution is added, and with alum solution a yellow color appears.

All of the above-described reactions are characteristic of the flavone derivatives, according to Onslow and to the Official Methods of the Association of Official Agricultural Chemists (1930).

The alcoholic solution of the crystalline material gives pink to red reduction compounds when HCl, mercury, and magnesium powder are added. This color reaction Shibata, Nagai, and Kishida (1916) state is characteristic of the flavone derivatives.

Upon prolonged hydrolysis of an aqueous solution of the recrystallized material with dilute HCl, a deep-yellow, feathery substance is thrown out of solution. This substance appears as very long, needle-like crystals under the microscope. The crystals so formed may be separated from the mother liquor by filtration and washed several times with hot water. They then give all the color reactions for flavone derivatives, as mentioned previously, but differ from the original unhydrolyzed material in that they are soluble in ether and cold alcohol but insoluble in hot water. This characteristic of the flavone or flavonol pigments in the non-glucoside state is mentioned by Onslow (1923).

The filtrate reduces Fehling's solution and gives a positive Bial's reaction for pentose sugar. In this connection, it should be mentioned that aqueous solutions of the original crystals failed to reduce Fehling's solution previous to acid hydrolysis.

Thus, the crystals occurring in home-canned green aspargus give the reactions characteristic of flavone derivatives. They react to hydrolysis by dilute acid with the splitting off of a reducing sugar, and the precipitation of a crystalline substance, insoluble in hot water but soluble in ether and cold alcohol, which still exhibits properties common to flavone derivatives. Hence, the conclusion seems warranted that this crystalline deposit is a flavone or flavonol glucoside, and is further substantiated by the fact that Nakaoki (1932) has been able to demonstrate the presence of a glucoside of the flavone series in fresh asparagus.

The appearance of this glucoside as a crystalline deposit in home-canned green asparagus may result from its extraction during heat processing and subsequent crystallization when the liquid in which the asparagus is cooked again cools to atmospheric temperatures.

It is interesting to note that this deposit, while occurring rather frequently in home-canned green asparagus, is found rarely in the commercially canned material. The reason for this possibly lies in the fact that under commercial conditions asparagus is harvested in a young state and is processed immediately, while under home-canning conditions the asparagus may be cut at an older stage or may be held for some time before processing. During this time, significant changes in chemical composition may take place.

At present, investigations are under way to determine more definitely the factors responsible for the appearance of the yellow material in home-canned asparagus.

There does not appear to be any ground for supposing that this material has significance from the public-health viewpoint.

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CEREAL FLOURS AS ANTIOXIDANTS IN DAIRY PRODUCTS[1]

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One of the major flavor defects in milk and some of its products is an oxidized flavor. It is variously designated as storage, cappy, cardboard, metallic, oxidized, and old ingredient. Milks vary considerably in their susceptibility to this flavor defect; and the effects of such factors as metallic contamination, light, and acidity have repeatedly been called to the attention of the dairyman. Occasionally the oxidized flavor develops in the milk, however, in spite of the fact that copper contamination has been excluded and exposure to light reduced to a minimum. Elimination of unusually susceptible milk may not be feasible because this would eliminate a large amount of milk from the better dairies. Therefore, other measures for controlling the oxidized flavor are receiving consideration. Attention is called to the addition of antioxidants to milk and various dairy products. The use of finely milled oat flour for the prevention of an oxidized flavor in ice cream and butter has been studied by several investigators: Corbett and Tracy (1937), Dahle and Josephson (1937a, b, c), Koenig (1937), Maack and Tracy (1938), Mueller and Mack (1937), and Peters and Musher (1937). The authors are not aware of published data on the value of other cereal flours as antioxidants in dairy products.

The object of this study was first to determine the effect of various cereal flours on the flavor of milk and some of its products, and second to find how these cereal flours affect some of their physical properties. In the present study the cereal flours were added to milk because it is a convenient medium for measuring the effectiveness of antioxidants in preventing the development of an oxidized flavor in dairy products as a class. However, the authors do not recommend the addition of cereal flours to market milk and they recognize the question of legality of the practice. The following cereal flours were studied: finely milled oat, whole oat, dry-milled corn (maize), bleached wheat, whole wheat, barley, rye, and rice.

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CEREAL FLOUR AND WATER SOLUTIONS

The cereal-flour solutions were prepared by adding four grams of flour to a volumetric flask and making up to 100 c.c. with distilled water. The solutions were heated to 63°C.(145°F.) for 30 minutes and then cooled to 46°C.(115°F.). Before filtering with suction, the solutions were centrifuged in a Mojonnier fat and extraction flask, thus removing a large amount of the undissolved material from the solution. This procedure greatly facilitated the filtration of the solutions.

Solubility, pH, and titratable acidity were determined and the results are shown (Table 1). Solubility was measured by determining the total solids in 25 c.c. of the filtrate. The solubility ranged

TABLE 1

Solubility of Several Cereal Flours in Water and the pH and Titratable Acidity of Their Solutions [1]

Cereal flour	Solubility [2]	pH [3]	Titratable acidity [4] .1 N NaOH
	pct.		*c.c.*
Oat (finely milled)	10.3	5.87	.40
Oat, whole	18.9	6.02	.85
Corn (maize)	11.5	5.87	.30
Wheat, bleached	29.9	5.82	.20
Wheat, whole	28.7	5.88	.45
Barley	15.0	5.85	.80
Rye	62.5	6.01	.40
Rice	3.6	5.91	.20

[1] Four grams of flour made up to 100 c.c. with water. [2] See text for method used to determine solubility. [3] Using quinhydrone pH meter and gold electrode. [4] A 25-c.c. sample was titrated.

from 3.6 per cent for rice flour to 62.5 per cent for rye flour. As will be shown later, there is no correlation between the solubility of the various flours and their antioxidative properties. The pH of all the flour solutions was close to 5.9, but the titratable acidity varied considerably for the various cereal flours.

CEREAL FLOURS IN WHOLE MILK

The milk used in this study was known to develop a typical oxidized flavor within 24 hours when approximately 4.5 p.p.m. of copper were added before pasteurization. One and twenty-five hundredths grams of cereal flour were added to 250 grams of milk which was then pasteurized at 63°C.(145°F.) for 30 minutes. After cooling, 250 grams of raw milk were added, thus making the final concentration of cereal flour .25 per cent. The milk was filtered twice

through a vacuum sediment tester while at a temperature of 18°C. (64°F.). The filter disk was cut from a cotton filter-strainer cloth. After holding for 24 hours at 5°C.(41°F.), viscosity, titratable acidity, and pH were determined. The milk was also scored for flavor by three or four qualified judges after periods of one, two, and three days. The results show that the various cereal flours had no significant effect on viscosity, titratable acidity, and pH of the milk but did affect the flavor of the milk to some extent (Table 2). Beginning with the second day the relative antioxidative properties of the various cereal flours became apparent. The two oat flours and corn flour had approximately equal antioxidative properties while the remaining cereal flours seemed to be lacking in them. The lower flavor scores for the whole oat and corn flours, when compared with finely milled oat flour, were due mainly to a more pronounced flour taste in the former.

CORN FLOUR AS AN ANTIOXIDANT FOR ICE CREAM

Since only the oat and corn flours seemed to have significant antioxidative properties in milk, the other cereal flours were not considered for further study. Although whole oat flour has antioxidative properties, the oat hulls eliminate it as a suitable ingredient for dairy products.

In this study, corn flour was compared with finely milled oat flour. The mixes (Table 3) were prepared, following the usual commercial procedures. The corn and oat flours were added to the mix with the sugar before pasteurization.

In a previous publication by Mueller and Mack (1937) it was shown that the whipping ability of ice cream mixes is lowered by the addition of finely milled oat flour unless a change is made in the stabilizer content so as to prevent over-stabilization of the mix, with a consequently high viscosity. When the gelatin content was lowered from .35 to .25 per cent and .5 per cent oat flour added, the viscosity and whipping ability of the mix was affected but little (Table 3). This, however, was not true when an equal percentage of corn flour was substituted for the oat flour (Table 3). Corn flour increased the viscosity of the ice cream mix to a greater extent than oat flour. The whipping ability of the ice cream mix was decreased and the melting resistance of the ice cream was increased when corn flour was substituted for the oat flour. No significant differences were noted in the titratable acidity of the mixes.

It is evident (Table 3) that both corn and oat flours are effective in retarding oxidized flavors in ice cream. Ice cream containing oat flour has a firmer body than ice cream without it. Furthermore, ice

TABLE 2

Various Cereal Flours as Antioxidants in Whole Milk

Cereal flour	Viscosity [1]	Titratable acidity	pH [2]	Flavor score [3] and criticisms					
				After 1 day		After 2 days		After 3 days	
	sec.	*pct.*							
Oat (finely milled)	22.3	.200	6.55	22.0	Slight flour or feedy	22.0	Slight flour or feedy	21.5	Slight cardboard, feedy
Oat, whole	21.8	.200	6.55	21.0	Flour	21.0	Flour	20.5	Slight cardboard, flour or feedy
Corn (maize)	21.8	.195	6.55	21.0	Flour	21.0	Flour	20.5	Slight cardboard, flour or feedy
Wheat, bleached	22.2	.197	6.56	20.0	Cappy, old	20.0	Slight cardboard, flour	19.0	Cardboard
Wheat, whole	22.0	.197	6.53	20.5	Cappy, mealy	20.0	Slight cardboard, flour	18.75	Cardboard
Barley	22.6	.195	6.55	19.5	Flour, old	19.5	Slight cardboard, flour	19.0	Cardboard
Rye	22.4	.195	6.54	21.0	Flour	19.5	Cardboard	17.5	Cardboard
Rice	21.8	.192	6.51	21.0	Papery, cardboard	19.25	Cardboard	17.0	Cardboard
Control—Whole milk	21.8	.195	6.51	21.0	Cardboard	19.0	Cardboard	16.0	Cardboard

[1] Time in seconds to flow from a standard pipette at 20°C.(68°F.). [2] Using quinhydrone pH meter and gold electrode. [3] 25 represents a perfect score.

TABLE 3

Effect of Finely Milled Oat and Corn (Maize) Flours on Certain Physical Properties of Ice Cream Mix,[1] and Quality of the Finished Product

Mix No.	Flour added to mix	Titratable acidity	Basic [2] viscosity	Overrun [3]	Drainage [4]	Flavor score [5] and criticisms after 3 weeks		Body and texture score [6] and criticisms after 3 weeks	
	pct.	*pct.*	*°M.*	*pct.*	*c.c.*				
1	0	.23	15.0	92	112	41.0	Old ingredient or oxidized	22.5	Slight fluffy and slight coarse
2	.5 oat flour	.24	12.0	95	184	42.5	Slight ingredient flavor	23.5	Slight coarse
3	.5 corn flour	.22	21.7	85	130	42.0	Slight ingredient flavor	23.0	Slight coarse and slight hard

[1] The mixes contained 14 per cent fat, 10 per cent serum solids, and 15 per cent sugar. No. 1 contained .35 per cent gelatin, while No. 2 and No. 3, with the flour added, contained .25 per cent gelatin. [2] At 20°C.(68°F.) using MacMichael viscometer with No. 26 wire. [3] After seven minutes in batch freezer. [4] From one pint of melting ice cream held at room temperature for 90 minutes. [5] 50 represents a perfect score. [6] 25 represents a perfect score.

cream containing corn flour has a firmer body than that containing oat flour.

CONCLUSIONS

1. The results secured in this study indicate that finely milled oat flour, whole oat flour, and corn flour have similar antioxidative properties when used in milk. Wheat, both bleached and whole, barley, rye, and rice flours possess but little antioxidative value in milk.

2. There was no direct correlation between the solubility of the various cereal flours and their antioxidative properties in milk.

3. Corn flour when used in ice cream has antioxidative properties similar to those of oat flour.

4. Corn flour is somewhat more effective as a stabilizer than oat flour. It increased viscosity of the mix and melting resistance of the ice cream and slightly decreased the overrun, when compared with oat flour.

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SURFACE DRYING OF FROZEN POULTRY DURING STORAGE[1]

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If market poultry is to be stored for periods of over a month it must be frozen in order to avoid spoilage from microbial activity, Lochhead and Landerkin (1935). In ordinary frozen storage one of the first forms of serious deterioration that occurs is the development of lighter-colored circular spots around the feather follicles and discolored areas of irregular shape on the skin surface. These markings are commonly referred to as "pock-marking" and "freezer-burn," respectively.

The results of Tressler (1935) and Moran and Wright (1937) have shown that surface drying is the primary cause of these defects. There is, however, little quantitative information available concerning the rate of drying at the temperatures and relative humidities commonly used for frozen storage. This investigation was undertaken to secure such information, and also to determine the value of different methods of packaging for preventing deterioration from surface drying.

FACTORS AFFECTING RATE OF DRYING

Although the principal factors determining the rate of drying are comparatively well understood, it appeared worth while to consolidate such of this information as is relevant to the drying of frozen products at this point. Briefly, the rate of drying of any product depends on two sets of conditions: first, the temperature, relative humidity, and movement of the surrounding atmosphere; and second, the rate of movement of moisture from the interior of the product to the evaporating surface. Obviously surface desiccation will occur whenever the removal of moisture from the surface is more rapid than its movement from the interior of the product to the surface.

Since most of the moisture in frozen products is in the solid state, its rate of movement to the surface is reduced to negligible proportions. Under these conditions surface drying can occur, and although the over-all loss of weight may be small, it may have a

[1] Issued as paper No. 29 of the Canadian Committee on Storage and Transport of Food.

serious effect on the appearance. In chilled (unfrozen) products the internal movement of moisture is not so seriously restricted, and greater over-all losses in weight can occur with less serious effects on the surface appearance. Obviously the surface desiccation of frozen products can be prevented only by reducing the rate of evaporation from the surface.

The drying of wet materials has been reviewed by Fisher (1935), who shows that the rate of evaporation is given by:

$$\frac{dw}{dt} = k(p_w - p_a)$$

where $\frac{dw}{dt}$ is the rate of evaporation; k a constant dependent in this case on the velocity and flow characteristics of the air; and p_w and p_a the vapor pressure of the evaporating water and that of the air, respectively. This equation has been found to apply under a wide variety of conditions, but it is not known whether it is strictly applicable to evaporation from a frozen product where most of the water is in the solid state. Since the quantity $(p_w - p_a)$ appears in most drying equations, however, it seems reasonably certain that the rate of evaporation from a frozen product is a function of this quantity.

Since the vapor pressure of water (p_w) and the moisture-holding capacity of the air (p_a) both decrease as the temperature is lowered, it is evident that the quantity, $p_w - p_a$, and the rate of drying will generally decrease as the temperature is lowered. At constant temperature p_w is fixed, but p_a will vary with the relative humidity. Thus the rate of drying can be reduced by lowering the temperature, increasing the relative humidity, or by a combination of these changes.

Moran (1934) places the eutectic temperature of the liquids in muscle in the region of —37.5°C.(—36°F.); and since most products start to freeze at about —1°C.(30.2°F.), it follows that at temperatures between these extremes, such as are normally used for frozen storage, the majority of the water will be present as pure ice or have an equivalent vapor pressure. It appears, therefore, that the vapor pressure of ice may be taken as a satisfactory estimate of p_w.

The value of p_a to be used in the drying equation is difficult to establish at freezing temperatures. Although ice is the stable state, Keyes and Smith (1934) point out that air appears to exert a solvent action, which becomes proportionately large at low temperatures, and retains a quantity of water vapor more closely related to the vapor pressure of supercooled water than to that of ice. Relative

humidity is defined in terms of the moisture contained in the air and not on the vapor tension. Since most metereological tables report relative humidity as usually defined, Ewell (1934), they represent the practical equivalent of vapor tension relative to that of supercooled water. Atmospheric vapor tensions computed on this basis could then exceed the vapor pressure of ice, and the drying equation would indicate condensation rather than evaporation at high relative humidities. For this reason it seemed preferable, at freezing temperatures, to express the vapor tension of the atmosphere as a percentage of the vapor pressure of ice at the same temperature. This quantity may be termed "humidity relative to ice," and at frozen storage temperatures this quantity may be 15 to 20 per cent higher than the corresponding relative humidities as ordinarily defined and computed.

EFFECT OF STORAGE TEMPERATURE AND HUMIDITY

In preliminary experiments frozen poultry was stored in closed containers over ice at temperatures of —13.5°C.(7.5°F.) and —22°C. (—7.5°F.) for periods of over a year. No evidence of surface marking appeared on any of these birds. This showed that freezer-burn and pock-marking were caused by surface drying, since it did not occur in a space having a vapor pressure equal to that of ice. Similar birds exposed in storage rooms at the same temperature but about 70 per cent relative humidity showed evidence of pock-marking, followed by freezer-burn, within two or three months. Since storage periods in excess of two or three months are usually desired, these results indicated that a study of the rate of drying at humidities between 70 and 100 per cent relative to the ice was of primary interest.

Temperatures of —13.5°C. and —22°C. were again chosen for the main series of experiments, since these are representative of the upper and lower part of the temperature range commonly used for storing poultry. These temperatures were maintained well within ±.5°C. throughout the greater part of the storage period. Occasional deviations of greater magnitude were of short duration.

Ice or calcium chloride solutions of appropriate concentration were used to obtain the desired humidities. The vapor pressure of calcium chloride solutions at sub-zero temperatures has been computed by Linge (1929) from known physical constants and thermodynamic considerations, while Awbery and Griffiths (1937) have reported experimental results. When the vapor pressures reported by these independent investigators were computed to humidities relative to ice, the two sets of values were in good agreement for

high humidities at temperatures just below the freezing point. As the humidity and temperature decreased, however, the values became divergent, the experimental results being as much as five per cent higher than the calculated values at a humidity of 85 per cent at —20°C.(—4°F.). On the basis of humidity relative to ice, both sets of data show that this quantity increases, for a solution of given concentration, as the temperature is lowered and naturally reaches 100 per cent at its freezing point. This reference point was therefore used for making the necessary extrapolations to obtain the vapor pressure of the solutions at the lower storage temperature, —22°C. These extrapolations could be made with greater assurance from Linge's data than from the experimental results. In consequence, all humidities reported subsequently were computed from Linge's values.

The initial concentration of the solutions was adjusted to give the following humidities relative to ice, at both storage temperatures: saturated solution (humidity unknown) 75, 80, 85, 90, 95, and 100 per cent (ice). The exact humidity of the atmosphere surrounding the product during the 83-week storage period was, however, subject to uncertainty arising from the following sources: (1) the divergence between calculated and experimentally determined vapor pressures as mentioned above; (2) dilution of these solutions with consequent increase in humidity during storage; and (3) the actual relative humidity of the air in the container which doubtless assumed some intermediate value between that corresponding to the vapor pressure of the product (ice) and the solution. During the storage period attempts were made to measure the relative humidity of the air in these somewhat small closed containers, but all of these failed to give sufficiently accurate results. Toward the end of the storage period a special hair hygrometer, calibrated over solutions of calcium chloride of known concentration and over ice, appeared to give reasonably reliable values at the higher storage temperature. At the lower temperature, —22°C., it was not sufficiently sensitive to give accurate readings.

In view of these considerations the reported humidities must be regarded as relative rather than absolute values. Nevertheless, it seemed desirable to form some estimate of the mean effective humidity to which the product was exposed. Although it was impossible to measure the concentration of the solutions during storage, this was done at the end of the experiment, and the corresponding humidity, relative to ice, computed. Although the rate of dilution doubtless decreased throughout the storage period, the total dilution was usually small, and the effective humidity was consequently taken as

the arithmetic mean of the initial and final humidities. Since the evaporating surfaces of the solution and product were of comparable magnitude, the relative humidity of the air was computed from the average of the mean vapor pressure for the solution, as discussed above, and for the product (ice). The only justification for making these computations is that they probably indicate the mean effective humidity more closely than either the initial or final humidities over the solutions. Actually the magnitude of both these corrections, and the uncertainty of the exact vapor pressure of the solutions decreases as the humidity increases. At calculated relative humidities of about 95 per cent or higher these are probably close estimates of the absolute value. Nevertheless, the relative nature of the observations must be recognized.

Three criteria were used for assessing the effect of the storage conditions on the product: (1) the time required for definite evidence of surface drying; (2) the percentage of the surface area affected at the end of the storage period; and (3) the moisture content of the skin. Since the containers were large glass desiccators, the product could be examined periodically without opening the vessels in order to determine the time required for surface marking to appear. At the end of the storage period the proportion of the skin area affected was estimated. The skin was removed while the birds were still frozen, and then ground and weighed at freezing temperatures for the moisture determinations. The moisture content was determined by drying *in vacuo* at 100°C.(212°F.) to constant weight. Two birds were stored under each set of conditions, and duplicate or triplicate measurements made on each bird to obtain the reported values.

RESULTS OF TEMPERATURE AND HUMIDITY EXPERIMENTS

The summarized results of this series of experiments (Table 1) give the storage temperature, the computed mean effective relative humidity throughout the storage period, and the observed relative humidity, measured with the special hair hygrometer, at the end of the storage period. These observed values are generally higher than the computed humidities. This is to be expected, since the maximum humidity would occur at the end of the storage period and could be higher than the mean effective humidity throughout the period.

The two birds used in each test seldom showed evidence of surface marking at the same time, nor were the proportions of the skin areas affected or moisture contents of the skins the same for the two birds at the end of the storage period. In order to give some

idea of the variability to be expected between different birds stored under the same conditions, the two individual values, rather than their mean, have been reported (Table 1).

The results indicate that at computed mean effective humidities of 95 per cent or lower, the product will show definite evidence of surface marking after two or three months' storage at both temperatures. Detrimental drying did not occur during 83 weeks' storage in a saturated atmosphere at —13.5°C.(7.5°F.) or at humidities

TABLE 1

Effect of Storage Temperature and Humidity on Surface Drying

Storage conditions			Time required for definite evidence of surface drying	Estimated proportion of skin area affected after 83 weeks' storage	Moisture content of skin
Temp.	Humidity relative to ice				
	Computed	Observed			
°C.	*pct.*	*pct.*	*wk.*	*pct.*	
—13.5	<82	93	8	40-60	21.4-21.8
	90	95	8-10	10-20	35.2-44.0
	92	96	8-14	20-25	32.2-33.4
	94	96	9-10	15-25	29.6-35.6
	96	97	22-24	5-10	42.8-46.8
	98	97	37	Slight	37.1-38.6
	100	100	>83	None	47.7-50.2
—22.0	<76	98	12-14	5-10	52.1-53.2
	89	99	8-9	5-10	43.0-48.6
	91	96	9-12	5-10	39.1-52.2
	93	98	22-26	5-10	48.4-50.1
	96	100 [1]	32-37	0-5	41.3-50.6
	98	100	>83	None	44.4-55.0
	100	100	>83	None	51.1-60.0

[1] Temperature variable when observations were made.

higher than 98 per cent at —22°C.(—7.5°F.). Humidities between about 95 and 98 per cent at —13.5°C. gave safe storage periods of six to nine months' duration. Conditions which caused surface drying to become evident in a relatively short time also caused severe desiccation, affecting a large proportion of the skin area, during long storage periods. At comparable humidities less deterioration occurred at —22°C. than at —13.5°C., a result in qualitative agreement with prediction from the drying equation given earlier.

It was expected that the final moisture content of the skin would constitute the best quantitative estimate of the deterioration from drying. Subsequently it was found that the moisture content of the skin of freshly killed poultry varied considerably, presumably as the result of varying fat content, since Holcomb and Maw (1934)

have shown that the moisture content of poultry muscle generally decreases as the fat content increases. It therefore seems probable that the final moisture content of the skin was dependent on the initial moisture content (not determined) as well as on the extent of drying under the various storage conditions. Since it was impossible to remove separately and quantitatively the skin from the affected and unaffected areas, the analyses had to be made on the skin from the entire bird. Under certain storage conditions the over-all reduction in moisture content might be small and therefore difficult to distinguish from the initial variations in moisture content.

The results (Table 1) show that the lower values for the moisture content of the skin were generally obtained under the drier storage conditions. Nevertheless, the large differences observed between birds stored under the same conditions made it impossible to draw definite conclusions from inspection. Statistical calculations were therefore made to determine whether the observed differences

TABLE 2

Analysis of Variance of Moisture Content of Skin

Variance	Degrees freedom	Mean square
Between birds stored under same conditions	14	11.1548
Between birds stored under different conditions	13	85.0626[1]

[1] Indicates one per cent level of significance.

between the moisture content of the skin of birds stored under different conditions were significantly greater than the differences between birds stored under the same conditions. The results of such an analysis of variance (Table 2) show that the differences in the moisture content under different storage conditions were highly significant.

When freezer-burn reaches an advanced stage, as it did under certain of these storage conditions, the drying may affect the flesh in certain areas to an appreciable depth. The flesh from such areas, together with a similar piece of apparently unaffected muscle from an adjacent area on the same bird, was removed for moisture determinations. Typical results (Table 3) show that severe freezer-burn may cause a loss of about one-third of the moisture originally present in the muscle.

EFFECT OF METHOD OF PACKAGING

Since the frozen product has a vapor pressure equivalent to that of ice at the same temperature, it is evident that the application of an effective moisture-proof lining material in the package should

allow the air surrounding the product to rise to 100 per cent relative humidity, and thus prevent freezer-burn. Poultry boxes are usually lined with some form of paper, and the adjoining edges are ordinarily overlapped but not sealed. Although relatively moisture-resistant paper stocks are now commonly used as lining materials, it is difficult to see how these can maintain the high humidities required to prevent freezer-burn if left unsealed. Some experiments were therefore made to determine the value of sealing the lining materials.

As it was not the object of these tests to determine the relative value of the many moisture-resistant lining materials available, only

TABLE 3

Moisture Content of Flesh From Affected and Unaffected Areas

Bird No.	Moisture content	
	Affected area	Unaffected area
	pct.	*pct.*
19	52.3	73.4
30	54.2	71.2

three paper stocks were used. One of these was parchment, chosen as typical of the moisture-permeable materials; the second was a heavy waxed paper, taken as representative of the better type of moisture-resistant materials; and finally a heavy aluminum foil (.01-inch) solidly laminated to a white sulphite paper. Since this thickness of aluminum foil is normally quite free from pinholes, this lining material was considered to be completely impermeable to water vapor.

The boxes with unsealed liners were packed in the usual way, allowing a generous overlap where the sheets joined. In the boxes with sealed liners the paper stock was placed in the boxes, and the joints on the bottom and sides were sealed on the inside with molten wax before the birds were packed. After packing, the lining material was folded over the birds and sealed in a similar manner on the outside. All tests were made on duplicate boxes of poultry.

The hair hygrometer, previously mentioned, was used to determine the relative humidity inside the liners during the storage period. In order to accommodate the sensitive part of this instrument, a perforated brass tube was inserted in each end of all boxes at the time of packing. This tube was secured in, and flush with, the end of the box on the outside. Where it passed through the liner, the latter was adequately sealed both to the tube and to the end of the box. The end of the tube was stoppered on the outside when the hygrometer was removed.

When packed, the poultry was frozen and stored in a room at —13.5°C.(7.5°F.), a temperature at which freezer-burn usually becomes evident in a few weeks. The relative humidity in this room was 69 ± two per cent except for short periods when it may have been somewhat higher. The storage period between packing and the final examination was about a year; but as it was impossible to pack and examine all of these boxes at one time, the storage period for individual boxes varied from 47 to 59 weeks.

The relative humidity in the boxes was observed periodically throughout the storage period. Since the hygrometer usually required 48 hours or more to reach equilibrium at this temperature, as well

TABLE 4

Effect of Method of Packaging

Material used and method of lining	Observations during storage		Final observations		
	Time after freezing for relative humidity to reach 95%	Estimated safe storage life	Number of weeks' storage	Relative humidity	Proportion of area affected
	wk.	*wk.*		*pct.*	*pct.*
Parchment, unsealed	0-1	8-9	48-57	75-77	55-68
Parchment, sealed	0-7	8-15	48-59	75-85	45-57
Waxed paper, unsealed	1-10	9-18	55-58	95-99	12-22
Waxed paper, sealed	56+	64+	47-56	98-100	2-12
Aluminum foil on sulphite stock, unsealed	28-48	36-56	47-53	82-95	8-10
Aluminum foil on sulphite stock, sealed	48+	56+	47-48	100	0-1

as frequent calibrations, it was only possible to obtain one observation per box per month on the average.

RESULTS OF PACKAGING EXPERIMENTS

In all of the boxes the relative humidity reached about 100 per cent during freezing and then fell off gradually during storage. These results have been summarized (Table 4) by reporting the time required for the humidity to fall to 95 per cent and also the final relative humidity just before the box was opened for examination. Since the results (Table 1) show that surface marking will usually occur after about eight weeks' storage at —13.5°C. at relative humidities below 95 per cent, the safe storage period for each method of lining was estimated by adding eight weeks to the period required for the humidity to reach 95 per cent. This value is also reported (Table 4) together with the number of weeks each box was

stored, the final relative humidity, and the proportion of the skin area affected when the final examination was made. In most cases two values are reported to give some indication of the observed differences between the duplicate boxes.

It is evident from the results that the several criteria used for estimating the quality of the liner—the estimated safe storage life, the final relative humidity, and the proportion of the skin area affected—are generally in good agreement. The results also confirm the findings of the previous experiment by showing that relative humidities of 98 per cent or higher are necessary to reduce freezer-burn to small proportions over a one-year storage period at —13.5°C.

With reference to the three materials used as liners it is evident that freezer-burn was most severe within the moisture-permeable parchment, less within the relatively impermeable waxed paper, and least within the impermeable aluminum foil. This result was to be expected, but it is of interest to note that a practical test of this nature is capable of demonstrating the value of the impermeable aluminum foil over the relatively moisture-proof waxed paper.

These experiments were designed essentially to determine the value of sealing the liner. The results (Table 4) show definitely that sealing all three materials prolonged the safe storage life and reduced the proportion of the skin area affected at the end of the experiment. It is worth noting that sealing improves a moisture-permeable material, such as parchment. Again a sealed, wax-paper liner appears to be equally as good as an unsealed, impermeable liner, such as aluminum foil. This indicates that the full benefit of a relatively impermeable liner can be obtained only if it is sealed. The aluminum foil used in these tests, although desirable, would probably prove too costly in commercial practice. A lighter weight foil, however, might prove to be equally effective and economically feasible. Sealing the relatively, but not completely, impermeable stocks (such as waxed paper) now in common commercial use would improve their utility. The development of a package capable of being easily and effectively sealed in practice would appear to be justified.

SUMMARY

An experiment, involving the storage of poultry for 83 weeks at temperatures of —13.5 and —22°C.(7.5 and —7.5°F.) and several relative humidities, yielded results in qualitative agreement with drying equations obtained with other materials, namely, that the rate of evaporation varies directly with the temperature and inversely with the relative humidity.

The period required for surface desiccation to affect the appearance of the product, the proportion of the skin area affected, and its moisture content after storage, were used as practical estimates of the rate of drying. These criteria showed that humidities less than about 95 per cent at both storage temperatures were unsatisfactory as the product was seriously affected in from two to three months. Humidities of 98 to 100 per cent maintained the product in satisfactory condition, with respect to surface drying, during 83 weeks' storage. Humidities intermediate between these extremes gave safe storage periods of six to nine months' duration. At comparable humidities, surface desiccation was less severe at the lower temperatures. Severely freezer-burned muscle tissue contains only 50 to 55 per cent moisture, whereas normal tissue contains about 72 per cent.

It was found that sealing the joints in the paper liners commonly used in commercial poultry boxes was beneficial regardless of the moisture-permeability of the paper stock used, such as parchment, heavy waxed paper, and heavy aluminum foil. Only the sealed aluminum-foil liner prevented surface marking during storage for one year at —13.5°C. Sealed, moisture-resistant, but not impermeable, stocks, such as heavy waxed paper, were as effective for reducing deterioration from surface drying as an unsealed, impermeable-foil liner.

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FROZEN STORAGE OF POULTRY. II. BLOOM[1, 2]

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The term "bloom" is used to designate the fresh surface appearance of meats. Lack of bloom does not necessarily imply spoilage or loss of flavor, but the consumer generally prefers products with good bloom. Successful storage practice must therefore aim to preserve bloom as well as to prevent deterioration through spoilage.

With poultry both the over-all quality and the bloom shortly after slaughter may vary considerably between different birds and it seems probable that poultry from the various commercial grades may suffer a differential loss of bloom during storage. This study was undertaken not only to determine the effect of various storage conditions on the retention of bloom, but also to determine whether poultry of different initial quality suffered a differential loss of bloom during storage in the frozen state.

Since bloom may be affected by many factors and cannot be defined objectively, it is generally assessed subjectively from the color and opacity of the superficial layers. Since Griffiths, Vickery, and Holmes (1932) have reviewed this subject in connection with an extensive study of bloom in lamb carcasses, a detailed discussion is unnecessary here. Briefly, these investigators report that darkening of muscle by methemoglobin formation is unlikely to occur to any appreciable extent at storage temperatures below —10°C.(14°F.). Apparently fats are more likely to change color, since Vickery (1932) reports that yellowing of rabbit fat may occur at —10°C. and recommends temperatures of —14°C.(6.8°F.) or lower to prevent this change. Since poultry is ordinarily stored at temperatures below —12°C.(10.4°F.), on this continent, it seems unlikely that any loss of bloom would result from a direct change in either the muscle or fat pigments.

Griffiths, *et al.* found that the main cause of loss of bloom was an increase in the opacity of the superficial tissues, which indirectly affects the color of the visible tissue. During freezing an increased opacity was obtained when the *rate* of evaporation was high, but

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[2] This is the second of a series of papers on frozen storage of poultry. The first of the series—Cook, W. H., Surface drying of frozen poultry during storage—was published in Food Research **4**, 407-418.

during storage the loss of bloom was approximately proportional to the *extent* of evaporation.

The factors affecting the rate and extent of evaporation in relation to the surface desiccation or freezer-burn in frozen poultry have been discussed in an earlier paper in this series by Cook (1939b). If loss of bloom in poultry is determined by evaporation, as it is in frozen lamb, it is to be expected that loss of bloom and freezer-burn will be associated. Nevertheless, these two conditions must not be confused. The increased opacity, causing loss of bloom, results from a uniform evaporation from the entire skin surface, whereas freezer-burn results from the severe desiccation of irregular areas. Naturally the latter condition has a serious effect on the appearance and may therefore be said to affect the bloom. In these studies, however, the bloom was assessed only from the appearance of areas unaffected by freezer-burn.

EXPERIMENTAL PROCEDURE

The poultry used in these studies consisted of birds killed and packed in the laboratory and birds killed in commercial packing plants. Dressing methods employed included dry plucking, semi-scalding, and wax dressing, but no effort was made to determine the relative effects of these various methods on bloom. The birds were precooled for 24 hours, graded, and packed at a temperature near the freezing point. Most of the poultry was placed in the freezing rooms within 48 hours after slaughter. All of the product was frozen at the same temperature as that at which it was subsequently stored.

Since no suitable objective method has yet been devised for estimating bloom, the method of subjective scoring, described in a previous paper by Cook (1939a), was used. Five grades of bloom were defined and each allotted a whole number. As the breast was scored separately from the legs and other visible parts of the body, the summation of these independent scores resulted in a possible score of 120 points per box of 12 birds. The figures subsequently reported for box lots of poultry are expressed on this basis and represent the mean scores of two judges.

In the previous study, Cook (1939a), it was observed that the most serious source of error in scoring arose from time-to-time variations in the inspectors' judgments. An attempt was made in the present investigation to estimate this error by scoring certain boxes on two successive weeks at the beginning and end of the storage period, on the reasonable assumption that during short intervals little change in bloom was to be expected. The differences between these successive scores enabled the magnitude of the time-to-time variability of the judges to be estimated by statistical methods.

Unfortunately, duplicate inspections throughout the entire storage period could not be tolerated, since frequent opening of the boxes would have affected the humidity in the package, hence the rate of evaporation.

EFFECT OF HUMIDITY AND TEMPERATURE ON BLOOM

Two birds, not initially scored for bloom, were stored at each of a number of relative humidities at two storage temperatures, as described by Cook (1939b). After 83 weeks' storage, the breasts of the birds were scored, yielding the values reported (Table 1) which

TABLE 1

Effect of Humidity and Temperature on Bloom

Stored at —13.5°C. (7.5°F.) for 83 weeks			Stored at —22°C. (—7.5°F.) for 83 weeks		
Computed mean effective relative humidity	Mean bloom score (possible=5)	Proportion of skin area freezer-burned	Computed mean effective relative humidity	Mean bloom score (possible=5)	Proportion of skin area freezer-burned
pct.		*pct.*	*pct.*		*pct.*
90	2.00		89	3.25	
92	3.25	10 to 25	91	3.75	5 to 10
94	3.00		93	4.25	
96	3.00	5 to 10	96	3.75	.....
98	4.50	Slight	98	4.00	None
100	5.00	None	100	4.50	None
General mean at each storage temperature.....	3.48	.....	.....	3.92	Difference =.44

Necessary differences for significance

	5% level	1% level
Between mean of duplicates.....	.50	.65
Between general means at each storage temperature.....	.20	.28

are the mean scores for the two birds stored at each condition. It is evident that the bloom on the portion of the skin surface unaffected by freezer-burn decreased as the proportion of the freezer-burned area increased. In spite of the observed irregularities, some of which may be attributed to variations in the initial bloom of different birds, it is evident that loss of bloom is greatest under storage conditions that promote drying. Bloom is therefore retained best at high relative humidities and low storage temperatures, since these conditions reduce evaporation.

This conclusion was supported by the results of a poultry packaging experiment described in the first paper of this series—"Surface Drying of Frozen Poultry During Storage"—which was published in Vol. 4, pages 407 to 418 of *Food Research*. The birds were not scored

for bloom but it was observed that in packages which maintained relative humidities in the vicinity of 98 to 100 per cent, the product retained excellent bloom over a one-year storage period at —13.5°C. (7.5°F.). In packages having 85 per cent relative humidity or less, the skin area unaffected by freezer-burn was practically devoid of bloom. The bloom on the product contained in packages that maintained humidities between these extremes was rated "fair" or "good" when examined by practical poultry graders at the end of the storage period.

In order to determine the effect of storage temperature on bloom of poultry, one box of each of four commercial grades, to be described later, were stored at —13.5°C. and —22°C.(—7.5°F.) for a 27-week period. These birds were packed in 12-bird boxes in the usual manner, using unsealed liners of heavy waxed paper. All of the boxes stored at the higher temperature decreased in bloom more than those held at the lower temperature. The average decrease in the bloom score for all four boxes was 34 points at —13.5°C., and only eight points at —22°C. Freezer-burn was also more severe at the higher temperature. It appears therefore that the differential loss of bloom can be explained by the differential rates of evaporation at the two temperatures.

BLOOM IN RELATION TO QUALITY

To determine the relation between quality and change of bloom during storage, poultry of five Canadian commercial grades was used. These grades, in order of decreasing quality, are as follows: Milkfed A, Milkfed B, Selected A, Selected B, and Selected C. Birds having a white fat and skin are classed as "Milkfed," the letters A and B indicating the amount of fat, fleshing, and finish. Selected A birds are fat and well fleshed but, as their skin and fat have a yellow color, they are excluded from the "Milkfed" classes. The Selected B grade is well fleshed but lacks the fat and finish of the higher grades. Selected C, a still lower grade, includes birds that are excluded from the higher grades for many reasons.

All of the birds were graded by skilled inspectors before being packed in 12-bird boxes with unsealed, heavy waxed-paper liners. All subsequent scoring was for bloom only, the judges attempting as far as possible to exclude consideration of the other factors contributing to their quality as a whole. Similar periodic scorings were made at approximate monthly intervals during a storage period of about one year.

The results of these detailed examinations, covering some 20 boxes of poultry and storage periods that overlapped and extended over a two-year period, are summarized (Table 2). A few boxes

only of the Selected C grade were stored, and since the bloom scores allotted to different birds varied greatly, little confidence could be placed in the results obtained.

Before considering the results, it is necessary to obtain some estimate of the error and variability of the reported mean values. The variability in the inspectors' judgments between examinations was estimated from the scores alloted to a number of boxes on successive weeks at the beginning and end of the storage period. This quantity expressed as a standard deviation was found to be ± 5.86. To this must be added the variability in the bloom score between different boxes within the same grade. On the basis of the initial in-

TABLE 2

Loss of Bloom in Relation to Grade

Storage period	Bloom scores for various commercial grades (Canadian)			
	Milkfed A	Milkfed B	Select A	Select B
wk.				
0-10	116	111	111	104
10-20	112	106	106	101
20-30	96	101	91 [1]	96
30-40	98	86 [1]	85	87 [1]
47-56	92 [1]	84	72	69
Decrease in bloom score over storage period.....	24 [2]	27 [2]	39 [3]	35 [3]

[1] Significantly lower than initial score. [2] Five-per cent level of significance. [3] One-per cent level of significance.

spections this standard deviation was found to be ± 6.50. The values reported are therefore subject to a standard deviation of ± 8.76 arising from these two sources. The significance of the reported differences was tested, using this value and the number of degrees of freedom applicable to the comparison in question.

The initial scores awarded to the Selected C birds differed significantly from all the other grades, except the Selected B, but the differences between the initial scores allotted to the four highest grades were not statistically significant. The time required for the bloom to fall to a value significantly lower than the initial rating is indicated (Table 2). There is some suggestion that the bloom is lost more rapidly as the grade or initial quality decreases, but this cannot be stated with assurance, since the results with the Selected B grade indicate the reverse.

The decrease in bloom over the entire storage period is shown for each grade at the bottom of the table. These results are more conclusive since all are statistically significant. However, the decrease

on the yellow-colored "Selected" grades is much greater than on the white and better-finished "Milkfed" grades. Since it seems unlikely that a chemical change in the pigments could occur at a storage temperature of —22°C., the decrease in bloom must be attributed to an increase in opacity of the skin or subcutaneous layers as a result of evaporation. It appears, therefore, that a given amount of evaporation must alter the apparent color, or surface appearance, much more in poultry that is yellow or dark than in birds that are initially lighter colored.

SUMMARY

When dressed poultry is stored in the frozen state, the loss of bloom during storage depends mainly on the extent of evaporation. Low temperatures and high humidities tend, therefore, to preserve bloom. In a package lined with a sealed, water-resistant material, such as waxed paper, little deterioration of the bloom can be detected during 50 weeks' storage at —13.5°C.(7.5°F.), but if the liner is not sealed, serious deterioration occurs in from 20 to 30 weeks at this temperature.

Poultry of different grades stored in the standard wooden boxes with unsealed liners at —22°C.(7.5°F.) all showed a significant decrease in bloom over a storage period of about a year. There was some evidence that the initial bloom was retained longer on the higher grade birds. The over-all decrease in bloom during the entire storage period showed that the lighter-colored "Milkfed" grades suffered less in appearance than the more yellow "Selected" grades. It appears, therefore, that under the same storage conditions the initial color is as important as the over-all quality of the bird in determining the loss of bloom that will occur.

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FREEZING TEMPERATURE AS RELATED TO DRIP OF FROZEN-DEFROSTED BEEF

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There is some divergence of opinion among investigators regarding the relative importance of factors which influence the amount of drip that exudes from frozen beef upon being defrosted. As early as 1908, Richardson and Scherubel recognized the importance of thawing frozen beef slowly as a means of reducing the amount of drip. Kallert (1923) confirmed these observations. He also pointed out that drip may be lessened by freezing fast enough to prevent the separation of water from the muscle fibers. Cook, Love, Vickery, and Young (1926) studied factors which they associated with the amount of drip from defrosted beef. They concluded that increase in the rate of freezing appeared to be the most important factor in the reduction of drip. Moran and Hale (1932) state that the temperature of freezer storage is very important in regulating the amount of drip from defrosted beef and that increased drip occurs at the higher storage temperatures and at fluctuating storage temperatures.

Empey (1933) reported that rate of freezing had no definite influence on the susceptibility of beef muscle to drip. His experiments show that drip production is primarily a function of hydrogen-ion concentration, the least drip being associated with muscle tissue having a pH value of 6.3 or higher. Recently, Sair and Cook (1938) found that the maximum drip was obtained from beef having a pH value of approximately 5.2 and as the hydrogen-ion concentration decreased, the net drip decreased to zero at about pH 6.4.

Data reported by Sair and Cook (1938) indicate that as the period of time between slaughter and freezing of beef is increased the amount of drip decreases. Similar observations have been made by the authors in experiments to be reported in a later paper.

It becomes apparent that there are a number of factors which may influence the amount of drip, and in a study of one of these factors it is important that the effect of other variables be taken into consideration.

In the present investigation wholesale ribs and rib steaks of beef were frozen at practical freezing temperatures, then thawed directly after freezing for the purpose of determining the effect of the freez-

ing temperature on the amount of drip that exudes from the frozen product upon being defrosted. In addition, studies were made on the pH of the meat and on the micro-structure of the frozen and defrosted beef.

FREEZING TEMPERATURE AS RELATED TO DRIP IN DEFROSTED WHOLESALE RIBS

Paired wholesale beef ribs were frozen in still air on wood shelves at temperatures of —12.2°C.(10°F.), —23.3°C.(—10°F.), —34.4°C. (—30°F.), and —45.6°C.(—50°F.). Ribs cut from select carcasses

TABLE 1

Amount of Drip From Paired Wholesale Ribs as Related to Freezing Temperature

Number of pairs	Freezing temperature	Average drip	Mean difference	t[1]	P[2]
	°F.	pct.			
2	—50	1.44	.22	1.37	.40
	—30	1.22			
2	—50	1.60	.09	2.75	.23
	—10	1.51			
2	—50	1.68	.13	1.44	.39
	10	1.55			
2	—30	1.63	.06	0.26	.84
	—10	1.69			
2	—30	1.63	.11	1.37	.40
	10	1.52			
2	—10	1.64	.16	0.76	.59
	10	1.48			

[1] $t = \dfrac{\text{mean difference}}{\dfrac{\text{standard deviation of mean difference}}{\text{square root of number}}}$.

[2] A significant mean difference is represented by a probability (P) of .05 to .01 and a highly significant mean difference is represented by a probability of .01 or less.

were frozen on the fifth day after slaughter. One week later they were thawed on trays in closed tin containers at a temperature of 10°C.(50°F.). The drip was collected during a three-day period.

Samples were taken for the histological study of the frozen cuts. The frozen tissues were prepared for microscopical study in accordance with the method recently recommended by Koonz and Ramsbottom (1939) as being particularly useful in studying frozen tissues.

In accordance with this method, the frozen tissues were dehydrated and simultaneously fixed in a vacuum-dehydrating apparatus operating at —34.4°C. After being sufficiently dehydrated, the small pieces of tissue were placed in paraffin and subsequently sectioned at 14 microns, stained, and studied microscopically.

A histological study of preparations of the frozen beef showed that very little intrafiber freezing occurred when wholesale rib cuts, weighing approximately 23 pounds each, were frozen at a temperature of —45.6°C. At higher freezing temperatures, —34.4°C., —23.3°C., and —12.2°C., the size of the ice crystals outside the fibers increased with each rise in temperature. At —12.2°C. the ice crystals forced the muscle fibers into ridge-like formations, which is characteristic of slowly frozen tissue.

There was a definite gradation in the color of the frozen beef from dark to light with each drop of 20 degrees in freezing temperature.

The hydrogen-ion concentration of the meat was determined by the use of the Beckman pH meter with glass electrode and calomel electrode. The pH values for all 24 ribs were within the range pH 5.51 to 5.90. The difference in pH value of paired ribs frozen at different temperatures was less than one-tenth in each comparison.

From the data on the percentage of drip based on the frozen weight of paired ribs (Table 1) it will be observed that the differences in drip between right and left side ribs frozen at different temperatures were small and non-significant, when treated statistically according to Fisher's method (1930).

FREEZING TEMPERATURE AS RELATED TO DRIP IN DEFROSTED RIB STEAKS

A second experiment was conducted for a two-fold purpose: first, to procure a greater difference in the rate of freezing than was possible with bone-in ribs; and second, to determine if similar results would be obtained with large and small cuts of meat.

Steaks one inch in thickness and weighing approximately one-half pound were cut from the boneless rib roll or that part of the longissimus dorsi muscle located between the fifth and twelfth thoracic vertebrae of select carcasses weighing approximately 550 pounds. Nine steaks were cut from each roll. Adjoining steaks were frozen on hardwood trays at temperatures of —6.7°C.(20°F.), —23.3°C. (—10°F.), and —45.6°C.(—50°F.). One week later, the steaks were weighed, suspended in closed tin containers, and thawed at a temperature of 10°C.(50°F.). One and one-half days were allowed for drip collection.

A statistical analysis of the data on the percentage drip based on the frozen weight of the steaks (Table 2) shows that the mean differences in drip between paired steaks frozen at —6.7°C., —23.3°C., and —45.6°C. are highly significant.

TABLE 2

Amount of Drip From Paired Rib Steaks as Related to Freezing Temperature

Number of pairs	Freezing temperature	Average drip	Mean difference	t	P
	°F.	pct.			
9	—50	4.85	1.40	10.74	<.01
	—10	6.25			
9	—10	6.25	1.77	14.70	<.01
	20	8.02			
9	—50	4.85	3.17	17.61	<.01
	20	8.02			

In the large standard beef-rib cuts, the freezing temperature in no way determined the susceptibility of the muscle tissue to drip (Fig. 1). Rate of freezing, therefore, does not appear to be an im-

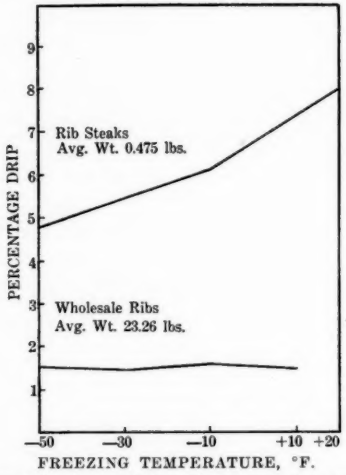

FIG. 1. Percentage drip from wholesale ribs and rib steaks frozen at different temperatures and defrosted at 50°F.

portant factor in the control of drip if the volume of beef is large in comparison with the area of cut surface of muscle tissue. On the other hand, if the volume of beef is small in comparison with the

area of cut surface of muscle tissue, then by increasing the rate of freezing the amount of drip can be materially reduced.

The hydrogen-ion concentration of the meat was determined by the method described in the preceding experiment. All values were within the narrow range pH 5.44 to 5.64. The average pH values for the meat were 5.52, 5.54, and 5.53, respectively, for the steaks frozen at —6.7°C.(20°F.), —23.3°C.(—10°F.), and —45.6°C.(—50°F.).

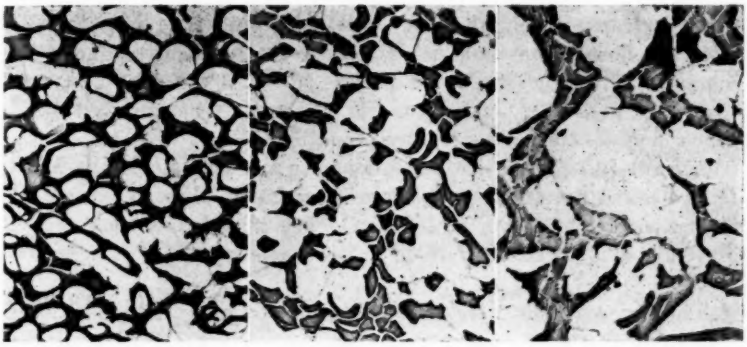

FIG. 2. Frozen at —50°F. FIG. 3. Frozen at —10°F. FIG. 4. Frozen at 20°F.

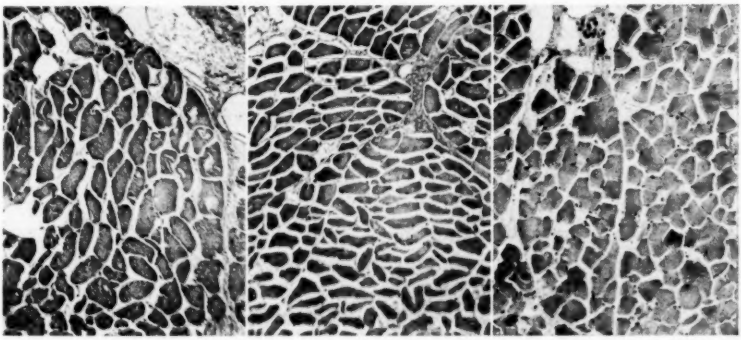

FIG. 5. Frozen at —50°F. and defrosted at 50°F. FIG. 6. Frozen at 20°F. and defrosted at 50°F. FIG. 7. Unfrozen.

Empey (1933) and Sair and Cook (1938) have reported that pH may be an important factor in regulating the amount of drip. The differences in drip obtained in the present experiment (Table 2) cannot be attributed to pH since the pH values for all steaks were very similar.

Histological studies were made on the frozen tissues prepared in accordance with the technique previously described. Histological

preparations were also made according to conventional methods for defrosted and unfrozen tissues.

Photomicrographs were taken with a Spencer microscope and Baush and Lomb type H photomicrographic camera. All photomicrographs (Fig. 2 to 7) have been magnified 83 times. Figures 2, 3, and 4 show the structural appearance of beef frozen at —45.6°C., —23.3°C., and —6.7°C., respectively. It will be observed that when steaks are frozen at —45.6°C. intrafiber freezing takes place. The water is frozen within the fibers as single columns of ice (Fig. 2) with the fiber protoplasm being located at the margin of the ice columns. When the steaks are frozen at —23.3°C. (Fig. 3) water that freezes at this temperature is frozen external to the fibers, which may be termed extrafiber freezing. When beef steaks are frozen at —6.7°C., the ice formations are located outside the fibers (Fig. 4) and are fewer and larger in size than those occurring in the muscle tissue frozen at —23.3°C. The fiber bundles are forced into ridge-like formations which are characteristic of slowly frozen meats.

Photomicrographs were made of cross sections of muscle tissue from thawed steaks frozen at —45.6°C. and —6.7°C. It will be observed that irrespective of freezing temperature there is a remarkable similarity (Fig. 5 and 6) in the microscopical appearances of the defrosted meat and of the unfrozen muscle tissue (Fig. 7).

SUMMARY

The data in these experiments show that irrespective of freezing temperature there was little drip in the large rib cuts where the area of cut surface was small in relation to the volume of meat. In the small steaks, where the area of cut surface was large in relation to volume of meat, the amount of drip was dependent to a larger extent on the freezing temperature. In large cuts the muscle tissue has opportunity to reabsorb the "frozen out" water, while in small cuts the fluids may be more readily lost by the tissue as drip.

When small steaks are rapidly frozen, intrafiber freezing occurs (Fig. 2); and when defrosted, the fluids will be retained for the most part by the fibers and the drip will be relatively small. If the steaks are slowly frozen, extrafiber freezing takes place (Fig. 4); and upon being defrosted, more of the fluid (Fig. 1) will be lost as drip before it can be reabsorbed by the partially dehydrated muscle fibers.

Photomicrographs show that, other factors being equal, the temperature of freezing materially affects the structural appearance of the frozen muscle tissue. Regardless of the temperature of freezing,

however, the histological appearance of the defrosted muscle tissue is quite similar to that of unfrozen muscle tissue.

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FROZEN STORAGE OF POULTRY. III. PEROXIDE OXYGEN AND FREE FATTY ACID FORMATION[1]

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In earlier papers in this series by Cook (1939a, 1939b) it has been shown that physical changes, principally surface desiccation, may cause depreciation of frozen poultry stored over a period of a few months. These changes, however, primarily affect the appearance rather than the flavor or eating quality. Complaints of "storage" flavor are sometimes heard in connection with poultry that has been held in the frozen state. It seems probable that this may originate from chemical changes in the fats owing to oxidation and hydrolysis. Analyses were therefore made to determine the free fatty-acid and peroxide-oxygen values of fat from frozen poultry that had been stored under various conditions.

Lea (1934) has studied the oxidation and hydrolysis of the skin fat of poultry stored in air and carbon dioxide at chill temperatures, i.e., 0 to —1°C.(32 to 30.2°F.). His results suggest that poultry fat is comparatively stable toward oxidation and that the hydrolysis of the fat is negligible if the growth of microörganisms is reduced.

ANALYTICAL PROCEDURE

The free fatty-acid content was determined by a slight modification of the method mentioned by Lea (1933a). Two grams of fat, dried and extracted by a procedure to be subsequently described, was refluxed with 25 ml. of neutral ethyl alcohol for four minutes and titrated with .05N potassium hydroxide, using phenolphthalein as the indicator.

The determination of peroxide oxygen is based on the oxidation of potassium iodide by the active oxygen in the fat to liberate iodine. The results are expressed as the number of ml. of .002N sodium thiosulphate required per gram of fat to react with the liberated iodine. A preliminary investigation was made on chicken fat to compare Lea's (1929, 1931) procedure with the modification suggested by French, Olcott, and Mattill (1935). It was found that both gave essentially the same results. Since the latter procedure

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requires less manipulation, however, it was used in all subsequent determinations.

Lea (1931) has pointed out that there are two inherent errors in this method. Although the amount of iodine liberated is determined by the peroxide oxygen present, that available for the titration depends first, on the quantity of fat present and second, on its degree of saturation. With a given fat the amount of iodine removed by absorption will be roughly proportional to the amount of fat in the solution. Likewise the amount of iodine available for the titration will be decreased more by a highly unsaturated fat than by a more saturated one.

Usually the peroxide-oxygen values reported in the literature are not corrected for these two errors and are consequently lower than the actual values. Since poultry fat is rather unsaturated (Lea 1934), it seemed desirable to determine the approximate magnitude of the error involved. This was done in a preliminary experiment by titrating solutions of various concentrations of the fat, plotting these values against concentration, and extrapolating to zero concentration. The results obtained with five fats, varying in peroxide-oxygen content from 4.8 to 43.4 ml. .002N sodium thiosulphate per gram of fat, showed that the actual values were about 20 per cent higher than the observed. This correction was not applied to the results of subsequent experiments, since these could only be interpreted on a relative basis and consequently would be unaffected by any constant percentage error.

EFFECT OF METHOD OF DRYING AND EXTRACTION

Since the peroxide-oxygen content of a fat may increase during its extraction from the product, some attention was given to methods that would minimize this change. The samples of fat obtained from the skin and subcutaneous fat layers of the frozen birds were chopped up as finely as possible in the freezing chamber in which the product had been stored. Little or no change in the peroxide-oxygen level could therefore have occurred at this stage.

The three following procedures were tested for drying this material and extracting the fat:

Procedure 1. Dried *in vacuo* at 50°C.(122°F.) followed by—A. Soxhlet extraction in a darkened room at 4.5°C.(40°F.), B. Soxhlet extraction in laboratory (diffuse daylight) at 25°C.(77°F.) (approx.).

Procedure 2. Mixed with anhydrous sodium sulphate, allowed to stand in the dark, and extracted as under A and B above.

Procedure 3. Mixed with anhydrous sodium sulphate, and petroleum ether added immediately so that the drying and extraction proceeded simultaneously during standing in a darkened room at 4.5°C.

Three such experiments were made, using three different poultry fats and drying periods varying from one to three days. The petroleum ether used for extraction was redistilled and tested for the presence of oxidizing substances. After extraction for approximately three hours, the petroleum ether was removed by heating on a steam bath followed by heating *in vacuo* at 50°C.(122°F.).

The results of these experiments (Table 1) indicate that in order to decide on the best procedure it is necessary to assume that the method giving the lowest values caused the least alteration in the fat. Although the initial peroxide-oxygen content of the fatty material used in the first experiment was zero with all drying and extraction procedures, it is evident from the results that the extracted fat prepared by Procedure 2 was less stable than that prepared by the other two procedures. The fatty material used in the second experiment contained peroxide oxygen immediately after extraction. In this instance the sample prepared by Procedure 2A had the minimum value, while that prepared by Procedure 2B yielded the highest peroxide-oxygen values observed. Although there is little to choose between Procedures 1 and 3, the indications are that Procedure 1 is superior. The fat used in the third experiment had a slight but definite peroxide-oxygen content. All three procedures gave essentially the same results.

On the whole the results indicate that the method of drying used in Procedure 2 is inferior to the other two tested. Although the results (Table 1) show some irregularities, in general, extraction in a darkened room at 4.5°C. yielded lower peroxide-oxygen values than that under ordinary laboratory conditions. In consequence the fat used in all subsequent determinations was obtained by drying *in vacuo* at 50°C. followed by extraction in a darkened room at 4.5°C.

EFFECT OF STORAGE CONDITIONS

The first series of experiments was comprised of analyses on fat obtained from poultry stored in commercial packages for various periods under different temperature conditions. The fatty material consisted mostly of the skin wherever it appeared to contain an appreciable quantity of fat. In the better grades, one bird usually yielded sufficient fat to make the necessary determinations. In the grades of lower quality, however, it was necessary to combine the fatty material from two birds to obtain sufficient fat. Duplicate

TABLE 1

Effect of Method of Drying and Extraction on Peroxide Oxygen[1] and Stability of Fat

Procedure	Method of drying	Extraction		Experiment 1. Fat A. Drying period 2 days			Experiment 2. Fat B. Drying period 1 day		Experiment 3. Fat C. Drying period 3 days
				Exposure period—days			Exposure period—days		Exposure period—days
		Method	Conditions	0	7	14	0	1	0
1	In vacuum oven at 50°C.(122°F.)	Soxhlet	In darkened room at 4.5°C.(40°F.)	0	0	1.0	6.5	31.0	1.5
	In vacuum oven at 50°C.(122°F.)	Soxhlet	In laboratory at 25°C.(77°F.)	0	0	1.2	7.3	28.0	2.2
2	Mixed with anhydrous sodium sulphate.	Soxhlet	In darkened room at 4.5°C	0	15	113	4.4	19.0	2.4
	Mixed with anhydrous sodium sulphate.	Soxhlet	In laboratory at 25°C	0	27	121	9.1	42.0	1.3
3	Mixed with anhydrous sodium sulphate, petroleum ether added immediately.	Decanted at end of drying period	In darkened room at 4.5°C	0	0.2	1.6	8.1	11.0	2.0

[1] Given as ml. .002 N. $Na_2S_2O_3$ per gram of fat.

extractions were made on the fat from different birds under each storage condition, and duplicate analytical determinations were made on each extract (Table 2).

Several of the boxes were stored for one period at one temperature and then held for another in a room at —40°C.(—40°F.) before the analyses were made. Since the changes in the fats should be practically negligible at this low temperature, this phase of the stor-

TABLE 2

Effect of Storage Conditions on Peroxide Oxygen and Free Fatty Acid in Poultry Fat Packed in Commercial Boxes

Storage		Peroxide oxygen	Free fatty acid as oleic acid	Remarks
Temperature	Time			
°C.	mo.	ml. .002N $Na_2S_2O_3$ per gm. fat	pct.	
—13.5	6	0	0.42	5 to 20% of skin area freezer-burned.
—13.5	6	0	0.52	5 to 20% of skin area freezer-burned.
—13.5	12¾	2.77	0.94	0 to 5% of skin area freezer-burned.
—13.5	12¾	2.53	1.6	Rel. hum. in package = 98%.
—13.5	12¾	2.47	0.63	50 to 80% of skin area freezer-burned.
—13.5	12¾	2.06	0.70	Rel. hum. in package = 80%.
—13.5	21	1.01	0.38	20 to 25% of skin area freezer-burned.
—13.5	21 [1]	0.82	0.57	20 to 25% of skin area freezer-burned.
—22.0	6	0	0.93	No freezer-burn.
—22.0	7 [2]	0.45	0.96	No freezer-burn.
—22.0	10½ [3]	0.82	0.62	.....
—22.0	10½ [3]	0.89	0.51	.....
—22.0	15½	0.48	0.67	10 to 50% of skin area freezer-burned.
—22.0	15½	0.84	0.58	10 to 50% of skin area freezer-burned.

[1] Subsequently stored at —40°C.(—40°F.) for 6 months before analysis was made. [2] Subsequently stored at —40°C. for one year before analysis was made. [3] Previously stored for 6 weeks at —13.5°C.(7.5°F.) and subsequently for 6 months at —40°C.

age history is merely indicated under "remarks." The relative humidity of the atmosphere surrounding the product in the package was measured in only a few instances; however, the area of skin affected by freezer-burn is reported for most of the samples.

The results show that little or no peroxide oxygen was formed within six months at either —13.5 or —22°C.(7.5 or —7.6°F.). Longer periods of storage gave average values of about 2 and .75 ml. .002N sodium thiosulphate at —13.5 and —22°C., respectively. Although the results indicate that more peroxide-oxygen formation occurred at the higher than at the lower temperature, this cannot be stated conclusively, since the fat from birds stored for an extended

period of one temperature in some cases had less peroxide oxygen than others stored under the same conditions for shorter periods. It appears therefore that the fat of different birds varies in its susceptibility to peroxide-oxygen formation. Similarly the amount of free fatty acid varied from .38 to 1.6 per cent as oleic acid between different birds, but without evident relation to the storage conditions.

TABLE 3

Effect of Temperature and Humidity on Peroxide Oxygen and Free Fatty Acid in Poultry Fat

Storage			Peroxide oxygen	Free fatty acid as oleic acid
Temperature	Relative humidity	Time		
°C.	pct.	mo.	ml. .002N $Na_2S_2O_5$ per gm. fat	pct.
—13.5	<82 to 90	25	5.08	.75
—13.5	<82 to 90	25	8.16	.64
—13.5	92 to 94	25	8.18	.56
—13.5	92 to 94	25	7.19	.58
—13.5	96 to 98	25	4.46	.51
—13.5	96 to 98	25	6.48	.53
—13.5	100	25	2.18	.57
—13.5	100	25	1.54	.88
—22.0	<76 to 88	25	0	.60
—22.0	<76 to 88	25	1.90	.55
—22.0	91 to 93	25	1.36	.54
—22.0	91 to 93	25	0.85	.58
—22.0	96 to 98	25	1.00	.49
—22.0	96 to 98	25	0.87	.41
—22.0	100	25	0	.45

In the second experiment two birds were stored at seven relative humidities at both —13.5 and —22°C. for 25 months in order to obtain more definite results. The method employed for controlling the relative humidity has been described in an earlier paper by Cook (1939a). When the analyses were undertaken it was found that the fat from one bird was inadequate for the determinations. Had the two birds stored under each condition been used to obtain the necessary material, it would have been difficult to separate the effect of different storage conditions from the variation between birds. In consequence the birds stored at two successive relative humidities, starting with the lowest, were treated as a group, i.e., the fat from one bird stored at a certain relative humidity was mixed with the fat from a bird at the next higher humidity, in order to provide sufficient material for the analysis. In this way two similarly treated

birds were left for a duplicate extraction. This procedure gave less information on the effect of humidity but enabled the variable behavior of the fat of different birds to be evaluated.

The results of these experiments (Table 3), although somewhat irregular, indicate that peroxide-oxygen formation decreases as the storage temperature is lowered and as the relative humidity increases at constant temperature. In order to determine the significance of the observed differences the results were subjected to an analysis of variance. The variance attributable to experimental error was computed from the difference between duplicate titrations on the same extract, and that attributable to the difference between birds was computed from the two extractions made on birds stored under the same conditions. In the complete results of this analysis (Table 4)

TABLE 4

Analysis of Variance of Peroxide Oxygen Content of Poultry Fat

Variance attributable to	Degrees freedom	Mean square
Experimental error	15	0.0216
Difference between birds	7	2.694 [1]
Difference between storage humidities	6	13.133 [2]
Difference between storage temperatures	1	154.724 [2]

[1] Indicates one-per cent level of significance. [2] Indicates five-per cent significance.

it is evident that all of the possible differences are significant with respect to differences between birds. The effect of temperature exceeds that of humidity, which in turn exceeds the difference between different birds.

The results of the free fatty-acid determinations reported (Table 3) show that the fat from all of the birds was remarkably similar with respect to this constituent. Some slight variations are evident, but, since they were not related to the storage conditions, no statistical analysis was made.

CONCLUSIONS AND DISCUSSION

The results show that the free fatty-acid content of poultry fat after storage varies somewhat between birds, but is usually low, and shows no relation to the storage conditions at freezing temperatures.

The storage temperature is the most important factor determining the extent of peroxide-oxygen formation in poultry fat, the amount increasing with increase in the storage temperature. At constant temperature, low relative humidities accelerate peroxide-oxygen formation. This is presumably the result of surface drying or freezer-burn which exposes more of the fat to the air. These two

are the most important factors determining the condition of the fat of poultry following storage in the frozen state. However, it has also been shown that the fat from different birds varies in its susceptibility to oxidation.

Even after prolonged storage (25 months) at —13.5°C.(7.5°F.) the maximum peroxide-oxygen titration observed was only about 8 ml. of .002N sodium thiosulphate per gram of fat. Correction for iodine removal by the fat would increase this some 20 per cent, but such corrections are without significance since the point at which a rancid odor or flavor becomes evident in poultry fat is unknown. Lea (1933b) reports that bacon fat becomes rancid when the peroxide-oxygen titration reaches 8 to 10 ml., and suggests (1934) that even higher values may be required before rancidity can be detected in poultry fat. This, together with the fact that the extracted fats all appeared to be sweet, indicates that probably the fat of none of these birds would have been regarded as rancid. However, the incipient changes indicated by the higher peroxide-oxygen values might have caused a loss or change of flavor.

It is somewhat difficult to assess the commercial significance of these results. Since the fat from poultry stored for a year or more at temperatures of —13.5°C.(7.5°F.) usually showed peroxide-oxygen values of less than three ml., it is concluded that this fat is normally quite resistant to oxidation and that storage temperatures of —13.5 to —18°C.(7.5 to 0°F.) are quite adequate for preventing this change over the normal storage period. At these temperatures, however, high relative humidities are necessary to prevent direct depreciation from freezer-burn, as shown by Cook (1939).

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REDOX POTENTIAL INDICATORS IN QUALITY CONTROL OF FOODS

I. CORRELATION OF RESAZURIN REDUCTION RATES AND BACTERIAL PLATE COUNTS AS INDICES OF THE BACTERIAL CONDITION OF FRESH AND FROZEN FOODS[1,2]

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The use of chemical indicators as agents for the determination of sanitary quality is well known in the dairy industry. For many years methylene blue has proved a valuable reagent in this respect, and more recently resazurin has been proposed as a dye which is capable of utilization for similar purposes by Barrett, Rutan, and Keenan (1937), Pesch and Cimmert (1929), and Ramsdell, Johnson, and Evans (1935).

Tests which could be used by control officials and inspectors interested in the detection and segregation of other foods having abnormally high bacterial content would be more widely used if the time interval necessitated by bacteriological or involved chemical processes could be abbreviated. One of the advantages cited for resazurin in the determination of the sanitary quality of milk is the shorter time interval required for the test in comparison with that necessary for methylene blue reduction. A further advantage is that the former dye also undergoes a two-stage reduction which involves progressive color changes from blue, the original color (resazurin), to pink (resofurin) and then to absence of color (hydroresofurin). A wider use of this reaction in food examinations appears to be practicable.

EXPERIMENTAL PROCEDURE

The purpose of the experiments here reported was to make a series of comparative determinations of bacterial plate counts and resazurin reduction times on various foods to ascertain whether this dye had possible uses for the quality control of foods other than milk. These tests were made on hamburg steak, shelled eggs, crabmeat, lobster meat, fish, gelatine, green beans, peas, and other vegetables.

[1] Read at the 40th annual meeting of the Society of American Bacteriologists in San Francisco, California, August 31, 1938.

[2] Contribution No. 133 from the Department of Biology and Public Health, Massachusetts Institute of Technology, Cambridge, Massachusetts.

Preliminary experiments were conducted with a modification of the technique outlined by others for milk—Barrett, Rutan, and Keenan (1937)—using an aqueous solution of resazurin (Eastman). As the foods were not fluids, 10 grams of the material under investigation were placed in 99 ml. of sterile distilled water, together with 10 grams of sterile washed sand, in a glass rubber-stoppered sterile bottle and shaken in a mechanical shaker for 10 minutes.

Further procedures were varied in accordance with the particular material. Filtration or centrifugal separation of the larger particles in suspension was usually necessary to make a homogeneous infusion before adding the dye. Such treatment increased the time necessary for reduction somewhat but by such preliminary treatment more consistent results were obtained. The tests were conducted with 10 ml. of the food infusion prepared as above, to which .1 ml. of a .05 per cent solution of the resazurin dye was added.

The first tests were conducted in open test tubes, covered to exclude light which may cause reduction of the dye. It is possible to run the tests in the presence of air but more comparable results were obtained after suction filtration which removed much of the dissolved oxygen obtained in the earlier mechanical shaking of the sample in preparation for the test.

The addition of previously heated sterile liquid agar-agar to such tubes after the dye had been added, thus forming a solid substrate for the test, was found to limit the diffusion of oxygen to a very thin layer (.1-2 mm.) at the top of the tube. Unless this was done, the blue or pink layer at the top of the tube would mix with the rest of the contents of the tube when it was removed for examination and also to some extent while the tube was in the constant temperature bath. Since the color change from pink (resofurin) to colorless (hydroresofurin) is a reversible reaction, and may be reversed by the introduction of O_2, any mixing of the layers of varying oxygen tension might cause inaccuracies in the interpretation of the test.

Further experiments indicated that rubber-stoppered test tubes equipped with stop-cocks, enabling evacuation of the tubes, were helpful. The most convenient and satisfactory procedure was found to be the use of Thunberg tubes, which could be evacuated by use of a water pump to remove the air just previous to the addition of the dye. The use of Thunberg tubes and preliminary evacuation also cut down the time necessary for color reduction and made the conditions of the test more comparable.

Two temperatures were used for incubation during the earlier experiments, 30 and 37°C.(86 and 98.6°F.). Although the reduction

of the dye appeared slightly more rapid at the higher temperature, a constant incubation temperature of 30°C. was used in all the tests cited.

Several hundred tests on various food materials have been carried out, using resazurin as an indicator and simultaneously determining bacterial plate counts on nutrient agar after incubation at 20°C.(68°F.) for three days.

As the color of the dye is superimposed on the natural color of the food infusion, it was necessary to keep control infusion samples without the dye to check the final reduction or complete reduction of the resazurin. The intermediate stage, when the pink color of the resazurin was predominant, was relatively easy to determine after a little experience. Observations were made at least every 15 minutes and records were kept of the time when the change to maximum pink occurred as well as the time necessary for complete reduction. As our preliminary experiments indicated that this test appeared to be particularly applicable to hamburg steak and broken-out eggs, both of which sometimes exhibit very high bacterial counts, a considerable number of samples of these materials have been tested.

TESTS MADE ON HAMBURG STEAK

The results obtained with hamburg-steak samples (Fig. 1) indicate the relation between resazurin reduction time and bacterial plate counts. The time intervals necessary for both complete reduction and partial reduction to the red or pink color are plotted. The bacterial counts are shown on a logarithmic scale.

The samples tested were all obtained in local meat shops and in general the tests were made within a few hours of purchase. A few of the samples having somewhat lower counts were prepared by having beef rounds cut and ground at the time of purchase.

Inspection of the graph indicates a rather definite relation between the time required for reduction of the dye and the bacterial plate counts. The change to the pink stage of the dye was much more rapid than the complete reduction in the samples showing lower bacterial numbers and in general occurred within three hours, while total reduction of the dye sometimes required six hours. Total reduction of the dye in the very high-count samples sometimes took place in 30 minutes, although usually longer periods were necessary. The definite appearance of maximum pink color generally took place within 90 minutes in all samples having more than 10 million bacteria per gram.

No efforts were made to determine types of bacteria present but it is known that different types of bacteria influence the reduction

rate to a considerable extent. It is of interest to note that staphylococci in milk have been reported by Barrett, Rutan, and Keenan (1937) to exhibit a particular aptitude in this respect.

Statistical calculations indicate a high correlation between pink or red reduction time and bacterial plate counts, an observation which has been made by others using the resazurin test for milk.[3] With both the pink reduction time and complete reduction time it is obvious that the shorter the time required for the color change

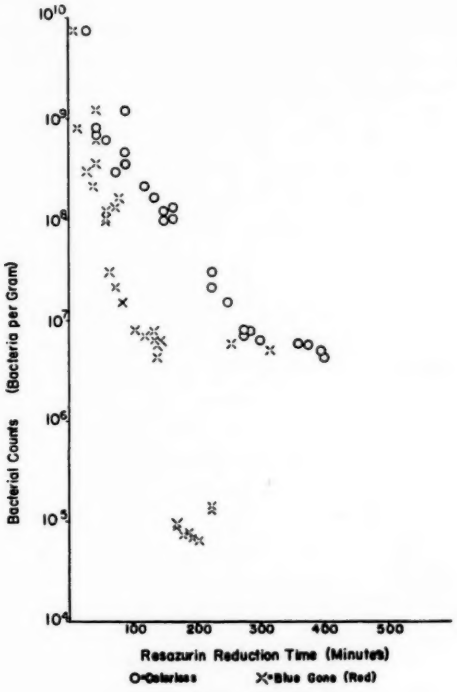

FIG. 1. Hamburg steak.

the higher the range of bacterial counts. Further data are now accumulating which make possible an extended statistical analysis of this apparent relation.

It is of interest that the only samples of hamburg steak we have examined having bacterial plate counts under one million bacteria per gram were commercially frozen hamburg samples bought on

[3] Personal communication with Dr. J. A. Keenan, Boston, Massachusetts.

the open market. These samples took about three hours for pink color development and over eight hours for complete color reduction.

TESTS MADE ON EGG PRODUCTS

The samples used for the resazurin test conducted on egg products included freshly broken-out egg yolks, freshly broken-out egg yolks and whites mixed, sugared yolks, and salted yolks. Results on egg whites were not included as the reduction time was much longer and the bacterial counts considerably lower than those shown (Fig. 2).

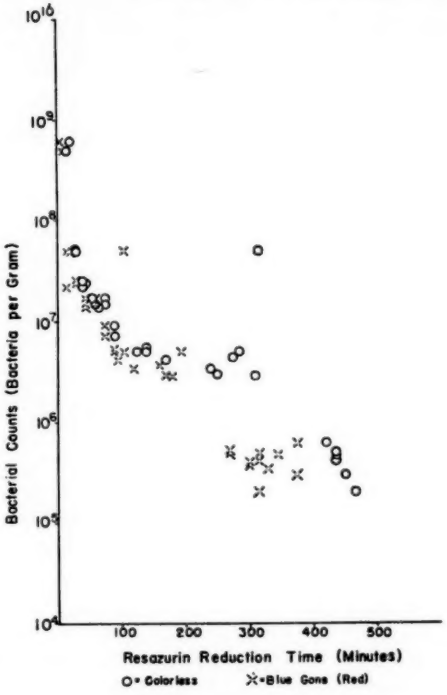

FIG. 2. Egg products.

There also appears to be a relationship between time of resazurin reduction and bacterial counts (Fig. 2). With the egg samples having highest bacterial contents both the pink reduction time and complete reduction time are somewhat shorter in comparison with those necessary for hamburg containing approximately the same bacterial content. There are some points in the graph which indicate that the reduction time may vary considerably in different samples

having bacterial contents at the same general level. Despite this fact it appears that this test may be used as a rough screen for the separation of samples of very high bacterial content in a fairly brief time, i.e., 15 minutes to a few hours, depending on the standards which one may set.

The same technique has been carried out on other food materials. The shortest reduction time noted with fish to date has been 108 minutes, with crab meat 60 minutes, with lobster meat 45 minutes, and with various vegetables 150 minutes. Numerous foods with low bacterial counts were found to require more than six hours for reduction, an interval which would probably lessen the value of this test for quality control purposes, although the time required is shorter than that necessary for bacterial plate counts.

CONCLUSIONS

The use of a modified resazurin technique has definite possibilities in providing a fairly rapid and reasonably accurate means of detecting certain foods containing abnormally high bacterial populations.

Extremely high bacterial counts may be detected in some foods in less than half an hour, especially if the intermediate stage of resazurin reduction is used as an index.

This method appears to be applicable to a number of foods, including hamburg steak and other comminuted meats, broken-out eggs, and other materials which may have high bacterial contents.

It is believed that the method should prove useful to food-control officials and those interested in quality control. Experiments now in progress in respect to its use in the check-up of successive stages of food-packing processes give indications of advantageous utilization in certain food industries.

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ANAEROBIC TECHNIQUE—A MODIFIED DEEP AGAR SHAKE

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Many methods have been proposed for cultivating anaerobic bacteria. In fact as early as 1887 a review of the older methods for cultivating anaerobes was published by Roux (1887). Since then many newer methods have been reported. Hall (1929) has summarized the various methods and discussed the different principles that are used in producing anaerobic conditions.

Any method for cultivating anaerobic bacteria will be used more frequently if it is simple, efficient, safe, and the equipment is inexpensive, particularly in laboratories that are limited in equipment and resources. This applies especially to work done in the public health and commercial fields where routine daily analyses are made for the detection of anaerobes.

A simple method, long recognized as a valuable procedure in anaerobiosis, is the deep agar shake in which oxygen-free environment is obtained by the deoxygenating effect of heat. However, this procedure has been restricted largely to isolation and qualitative techniques owing to difficulties in making accurate counts in a round tube.

EXPERIMENTAL PROCEDURE

The advent of the oval tube, the two broader sides of which are approximately parallel, enabled us to develop a modified and a more accurate deep agar shake method suitable for quantitative technique. As purchased these oval tubes[1] are difficult to plug, the agar has a tendency to wet the plug on agitation following inoculation, and a large surface is exposed for absorption of oxygen.

To overcome these difficulties we designed an oval test tube that has a round lip and neck as illustrated (Fig. 1). After filling the tube to the base of the neck with medium, it is sterilized. Prior to inoculation these tubes are heated in an Arnold sterilizer for ten minutes. After rapid cooling of the deoxygenated tubes to 40 to 45°C.(104 to 113°F.), they are quantitatively inoculated and then immediately immersed in ice water to solidify the inoculated medium rapidly. Promptly upon solidification the necks of the tubes are

[1] Listed as agar-slant tubes by Fisher Scientific Company.

partly filled (approximately two-thirds) with reduced methylene-blue agar, containing 1:20,000 parts of dye and two per cent dextrose, at pH 8.2 so that, following the work of Rittenberg, Anderson, and ZoBell (1937), the rate and depth of oxygen absorption can be determined.

This seal or "indicator cap" excludes oxygen from the inoculated medium during incubation, thereby increasing the accuracy of this

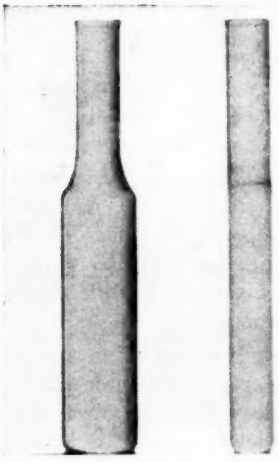


FIG. 1. Modified deep agar shake tube, front and side views. (Approximately two-fifths actual size.)

method by making available for anaerobic growth all of the inoculated medium. At no time during these studies was oxygen absorbed deeper than 15 mm. into the "indicator caps" during several days' incubation at 37°C.(98.6°F.). When incubated at higher temperatures for thermophilic anaerobes the oxygen absorption was less.

TABLE 1

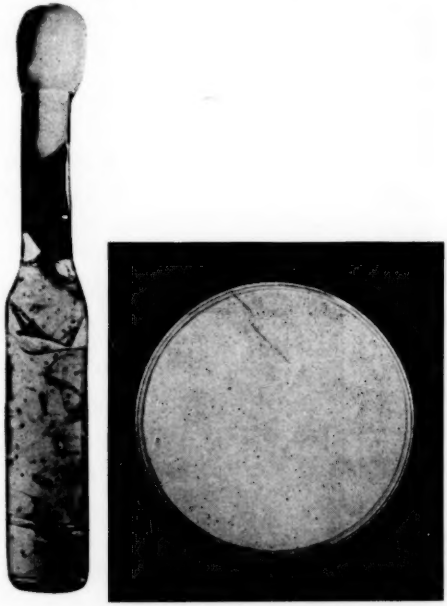
Comparative Anaerobic Bacterial Counts

Product tested	Weiss-Spaulding method	Modified deep agar shake	Spray method
Clostridium histolyticum[1]	77,000	70,000	0
Clostridium putrificum[1]	2,000,000	2,200,000	450,000
Digested milk	19,000	20,000	2,200
Dirt	34,000	30,000	3,000
Saliva	290,000	300,000	3,100
Polluted river water	500,000	400,000	70,000

[1] Broth culture.

Incubation at room temperature required about two weeks for complete penetration of the "indicator cap" by oxygen.

Series of tests were made using the above-described method and, in addition, two other comparatively simple methods, the Weiss-Spaulding (1937) and the Spray (1931), were used. It is of interest that each of these three methods employs a different principle in producing anaerobic conditions. It is of importance that the modified deep agar shake method is the only procedure reported in this



A B

FIG. 2. A—Modified deep agar shake tube seeded from *Clostridium putrificum* broth culture. B—Plate seeded from same dilution as tube, incubated anaerobically by Weiss-Spaulding method.

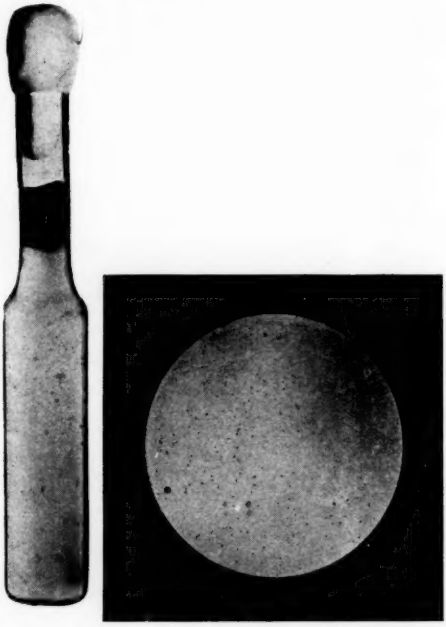
paper in which anaerobiosis is obtained immediately as shown by the reduction of methylene blue.

The materials that were tested included two cultures of anaerobic organisms secured from the American Type Culture Collection, namely *Clostridium histolyticum* and *Clostridium putrificum;* digested milk; dirt; saliva; sewage-polluted river water. After a series of preliminary experiments it was found that the medium which gave good growth for all materials tested was Bacto Tryptone Glucose Extract Agar. This was used as a nutrient medium throughout

these experiments. The anaerobic counts were determined and compared after incubation for 48 hours at 37°C.

DISCUSSION OF RESULTS

The data listed for comparison (Table 1) are representative, individual determinations taken from a series of tests made upon each type of product studied. We believe that, for present purposes, a better comparison is revealed by using actual counts rather than



A B

FIG. 3. A—Modified deep agar shake tube seeded from *Clostridium histolyticum* broth culture. B—Plate seeded from same dilution as tube, incubated anaerobically by Weiss-Spaulding method.

averages. In each group of tests the results showed the same trend and the best correlation was between the modified deep agar shake and the Weiss-Spaulding methods. These two methods also yielded the highest counts.

Even when gas was produced in the deep agar shakes the counts usually could be made satisfactorily (Fig. 2), as shown by results obtained with a culture of *Clostridium putrificum*. The colonies in the deep agar shake tube on the left can readily be seen and counted

even though the gas has broken the agar column. On the right is the same dilution of this culture that has been grown anaerobically by the Weiss-Spaulding method. Gas bubbles can be seen around several colonies on the plate.

Results obtained with *Clostridium histolyticum* that has been cultivated by the modified deep agar shake (left) and the Weiss-Spaulding method (right) made from the same dilution are given (Fig. 3). It is readily seen that with the broad-sided tube the colonies can be counted easily. The dark area at the top of the "indicator cap" shows the depth to which the absorbed oxygen has penetrated and that it has not reached the inoculated medium.

After testing the different methods for anaerobiosis on a variety of materials, in our hands the modified deep agar shake method proved to be the most satisfactory. This technique seems to be the most practical, especially in the public health and commercial fields where routine daily analyses are made. This is true because the modified deep agar shake method is simple, efficient, and accurate; no expensive equipment is needed; anaerobiosis is established immediately in the inoculated media; and there is no danger of explosion as is the case where hydrogen is employed to produce anaerobiosis.

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BACTERIA OF THE COLON-AEROGENES GROUP ON NUT MEATS

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Although nut-meat production and importation in the United States have progressively increased during the past decade, comparatively little attention has been given to the sanitary aspects of manufacture and distribution. Statistics compiled by the Bureau of Agricultural Economics, United States Department of Agriculture, (1938) show that while there has been considerable fluctuation in pecan production from year to year, a high peak of approximately 106 million pounds was reached in 1935. Preliminary figures for 1937 indicated a pecan crop of about 81 million pounds for that year.

With the growth of pecan production, various steps have been taken to familiarize the public with this product and the many ways in which it can be used. A survey of conditions surrounding the marketing of pecans, conducted by Jones and associates (1932) of the United States Department of Agriculture, and other investigations reported by the Bureau of Foreign and Domestic Commerce of the United States Department of Commerce have indicated an increased use in recent years of not only pecans (1931), but also filberts (1931), peanuts (1931), almonds (1932), and walnuts (1932). Whereas previously shelled nut meats were principally supplied to confectioners, bakers, and ice cream manufacturers, more recently there has been a growing demand from salters and retail distributors. This increased popularity of nut meats has, of course, resulted in a proportionate development in commercial shelling operations.

In considering the sanitary aspects of nut-meat production and distribution, attention is naturally directed to studies of the presence of *Escherichia coli* since this specific organism is frequently used as an index of fecal contamination. Its presence, therefore, on nut meats, which are often eaten raw, may indicate that such products so contaminated are potential sources of filth diseases. As a part of an investigation to determine the incidence of *Escherichia coli* on candy, Weinzirl (1927, 1929) explored the probability that nuts were an indirect source of such contamination, following his finding that pollution more often occurred in candies containing nuts than in other types of confections. Weinzirl assumed that nuts could become

contaminated in several ways, namely, through contact with soil, in the process of shelling, while being incorporated into candy, or while being dispensed. Adopting a laboratory procedure of introducing nuts into Durham fermentation tubes containing lactose broth, incubating for 24 and/or 48 hours at 37°C. (98.6°F.), streaking the positive presumptive cultures on Endo agar, isolating and identifying the colon-aerogenes organisms found according to the classification of Levine (1921), he examined 200 specimens, comprising 10 varieties obtained from wholesale distributors, and 400 specimens, comprising four varieties, obtained on the retail market. *Escherichia coli* was found in two per cent of the 200 specimens collected from wholesale distributors, and in .5 per cent of the 400 specimens gathered from retail stores. As these figures for nut meats were lower than those obtained in his investigation of candy, Weinzirl concluded that nuts were not necessarily the principal source of *Escherichia coli* on candy and that occurrence of such bacteria could be explained by contamination during handling. As a contrast to the findings reported by Weinzirl, Prucha (1936) and Tracy and Brown (1936) examined nut-meat samples secured at ice cream plants and found *Escherichia coli* in 50 per cent of the samples examined.

EXPERIMENTAL WORK

During the years 1934 to 1936 investigations were conducted by the authors to determine whether bacteria of the colon-aerogenes group are to be found on nut meats removed aseptically from the shell, the incidence of *Escherichia coli* on nut meats collected from commercial sources, the longevity of this organism on stored nut meats, and the frequency of biochemical colon-aerogenes types as they may occur on nut meats.

As pointed out by Tanner (1932), nut meats within the unbroken shell are considered to be sterile. In order to test the soundness of this assumption, 34 samples of nuts in the shell, representing four varieties, were washed in a weak solution of phenol, dried with sterile towels, and the meats removed from the shell aseptically. Bacteriological examination of the meats thus obtained failed to reveal the presence of members of the colon-aerogenes group in any of the samples examined. Fourteen samples contained no viable bacteria, and 20 samples contained from 10 to 800 bacteria per gram of nut meat.

Before beginning the examinations to determine the incidence of *Escherichia coli* on nut meats collected from commercial sources consideration was given to the development of a procedure which could be depended upon to yield the maximum number of colon-aerogenes bacteria present. Since the organisms sought, if present, are to be

found on the exterior of the nut meat, the basic procedure for the examination involved the preparation of washings obtained by thoroughly shaking known amounts of nut meats in known amounts of sterile water to provide material which could be used for inoculation.

After numerous experiments to ascertain the size of subsample that would yield maximum results and the combination of subsample and wash water that would best suit the purposes of the investigation, it was concluded that the procedure most productive of consistent results called for the washing of 50 grams of nut meats in 50 c.c. of sterile water. This combination was found to have advantages over that in which 10 grams of nut meats were washed in 100 c.c. of water. Therefore, in the examination of each sample col-

TABLE 1

Variety and Distribution of Samples of Shelled Nuts

Type of nut meat	Retail market	Domestic	Imports	Total
Almonds	22	11	16	49
Brazils	23	2	2	27
Cashews	24	0	39	63
Filberts	10	0	7	17
Jumbo peanuts	2	0	0	2
Pecans	35	117	9	161
Pignolias	12	0	0	12
Pistachios	0	0	5	5
Spanish peanuts	2	0	0	2
Black walnuts	24	22	0	46
English walnuts	46	82	36	164
Total	200	234	114	548

lected from commercial sources, 50 grams of nut meats were weighed in a sterile wide-mouth bottle, 50 c.c. of sterile water was added, the mixture was well shaken, and the washings thus obtained were used for the preparation of decimal dilutions for inoculation into .5 per cent lactose broth. Cultures from the lactose broth showing gas after 24 hours' and/or 48 hours' incubation at 37°C. were streaked on eosin methylene blue agar plates. Identifications of the members of the colon-aerogenes group were carried out according to procedures given in Standard Methods of Water Analysis (1933).

Using the above-described method 548 samples of nut meats, representing 11 varieties, were collected and examined. The samples were grouped according to origin into (1) retail market (200 samples), purchased at retail shops in Washington, D. C.; (2) domestic shelled (234 samples), collected aseptically in sterile jars from shell-

ing plants in the large producing areas in the United States; and (3) imports (114 samples), collected aseptically in sterile jars from shipments from nine foreign countries. The variety and distribution of samples is shown (Table 1).

DISCUSSION OF RESULTS

Retail Market Samples: The retail market samples were collected in paper bags as commonly dispensed over a counter. *Escherichia coli* was isolated from eight of the 10 varieties of nuts in decimal dilutions representing .01-gram portions, and in this amount was

TABLE 2

Incidence of Members of Colon-Aerogenes Group in Retail Market Nuts

Type of nut meat	Number of sub-samples	Number subs showing group [1]	Subs showing group [1]	Number subs showing *Escherichia coli*	Subs showing *Escherichia coli*
			pct.		*pct.*
Almonds	22	9	40	3	13
Brazils	23	8	34	1	4
Cashews	24	13	54	5	20
Filberts	10	4	40	1	10
Jumbo peanuts	2	0	0	0	0
Pecans	35	17	48	4	12
Pignolias	12	6	50	2	16
Spanish peanuts	2	1	50	0	0
Black walnuts	24	7	29	11	45
English walnuts	46	15	32	3	6
Total	200	80	....	30	....

[1] By "group" is meant members of colon-aerogenes group, exclusive of *Escherichia coli*.

present in 45 per cent of the black walnuts, 20 per cent of the cashews, 16 per cent of the pignolias, 13 per cent of the almonds, and 12 per cent of the pecans. Of the remaining five varieties, *Escherichia coli* was found in the Brazil nuts, filberts, and English walnuts in from four to 10 per cent of the samples examined. Examination of jumbo peanuts failed to show *Escherichia coli* or other members of the colon-aerogenes group, whereas in Spanish peanuts the group organisms exclusive of *Escherichia coli* were demonstrated. Nine of the ten varieties contained *Aerobacter aerogenes* and intermediates, and these were present in from 29 to 54 per cent of the samples (Table 2).

Domestic Shelled Samples: The products of 38 commercial shelling plants were sampled. These samples represented practically every stage in the shelling operation and included stored products and glass-packed and tin-packed material. Examination of these samples

demonstrated the presence of *Escherichia coli* in all but one of the five varieties of nut meats examined. This organism was present in 26 per cent of the pecans and 18 per cent of the almond samples. The highest incidence of *Escherichia coli* (68 per cent) was found in the black walnuts, which present the greatest problem in shelling and consequently require the maximum amount of handling. English walnuts, which are relatively easy to shell and which probably receive a minimum of handling, contained *Escherichia coli* in only six per cent of the samples. All five varieties contained other members of the colon-aerogenes group of bacteria, and these bacteria occurred in from nine to 50 per cent of the subsamples (Table 3).

TABLE 3

Incidence of Members of Colon-Aerogenes Group in Domestic Shelled Nuts

Type of nut meat	Number of subsamples	Number subs showing group [1]	Subs showing group [1]	Number subs showing *Escherichia coli*	Subs showing *Escherichia coli*
			pct.		*pct.*
Almonds	11	1	9	2	18
Brazils	2	1	50	0	0
Pecans	117	52	44	30	26
Black walnuts	22	8	36	15	68
English walnuts	82	16	19	5	6
Total	234	78		52	....

[1] By "group" is meant members of colon-aerogenes group, exclusive of *Escherichia coli*.

Import Samples: The import samples were collected from import shipments from eight countries in Europe and the Orient, and from Mexico. The examination of three varieties—almonds, cashews, and English walnuts—showed the presence of *Escherichia coli* in 62, 51, and 36 per cent respectively, of the samples. An insufficient number of samples of the remaining four varieties were examined to warrant any reliable conclusions on the computed percentages (Table 4).

SURVIVAL OF *ESCHERICHIA COLI* ON NUT MEATS

To aid in evaluating the significance of the results of these objective examinations, approximately two pounds of each of six varieties of nut meats were artificially infected with *Escherichia coli* in the following manner:

Using 24-hour agar slants, three liters of a turbid suspension of the test organism were prepared in tap water. Approximately two pounds of nut meats were immersed in the suspension and mixed by stirring constantly for approximately 15 minutes. Smaller lots, con-

sisting of 100 grams of each variety of nut meats, were similarly treated. The supernatant fluid was then drained off from both types of samples and the nut meats were spread evenly in open pans and dried at 56°C.(132.8°F.) until the 100-gram samples had returned to their original weight. Portions of the two-pound lots of nut meats were examined immediately to determine the extent of contamination, and the remaining portions were then stored at room temperature in tightly closed tin containers.

Examinations for the presence of *Escherichia coli* were made at intervals during a period of 80 days. The results of these examinations indicated that *Escherichia coli* could be detected under these

TABLE 4

Incidence of Members of Colon-Aerogenes Group in Import Nuts

Type of nut meat	Number of subsamples	Number subs showing group [1]	Subs showing group [1]	Number subs showing *Escherichia coli*	Subs showing *Escherichia coli*
			pct.		*pct.*
Almonds	16	4	25	10	62
Brazils	2	1	50	0	0
Cashews	39	11	28	20	51
Filberts	7	1	14	1	14
Pecans	9	3	33	4	44
Pistachios	5	1	20	2	40
English walnuts	36	11	30	13	36
Total	114	32	....	50	....

[1] By "group" is meant members of colon-aerogenes group, exclusive of *Escherichia coli*.

conditions for approximately 68 days. This experiment was repeated with approximately the same results. As an additional check on these results, seven routine shelling-plant samples examined five weeks after collection were found to contain *Escherichia coli* in dilutions representing .01-gram portions of nut meats.

FREQUENCY OF BIOCHEMICAL COLON-AEROGENES TYPES ON SHELLED NUT MEATS

To determine the frequency with which various biochemical types of the colon-aerogenes group occurred on the several varieties of nut meats, 102 samples, comprising 16 retail market samples; 54 factory samples; and 32 import samples were selected for more extensive examination from the 548 samples listed (Table 1). Lactose broth tubes were inoculated with 10, 1, .1, .01, and .001 c.c. of washings from a 50-gram subsample. All tubes showing gas in 24 and/or 48

hours were streaked on eosin methylene blue agar plates. As many colonies were fished from these plates as there were colony types. The cultures were then purified by plating on litmus lactose agar. Well-isolated colonies were chosen and tested for (1) indol production, (2) methyl red reaction, (3) acetylmethylcarbinol production, and (4) citrate utilization. Over 700 cultures were studied in this

TABLE 5

Frequency of Biochemical Types of Colon-Aerogenes Group on Nut Meats

Biochemical type	Indol	Methyl red	Voges-Proskauer	Citrate	Number of cultures	Per cent
Typical *Escherichia coli*	+	+	—	—	149	40.0
Atypical *Escherichia coli*	—	+	—	—	30	8.0
Typical *Aerobacter aerogenes*	—	—	+	+	161	44.0
Atypical *Aerobacter aerogenes*	+	—	+	+	11	2.7
Typical intermediates	+	+	—	+	5	1.3
Atypical intermediates	—	+	—	+	15	4.0

manner. After eliminating the duplicate cultures isolated from each dilution of washings from a given sample, 371 cultures remained. The frequency of biochemical types is given (Table 5).

Based on the presence or absence of the above-mentioned biochemical types, the samples were grouped as shown (Table 6).

TABLE 6

Presence or Absence of Biochemical Types on Samples of Nut Meats

Group	Number of samples	Per cent	Colon aerogenes types present
1	10	9.8	Only *Escherichia coli* present.
2	29	28.5	Only *Aerobacter aerogenes present.*
3	6	5.8	Only *Aerobacter aerogenes* and intermediates present.
4	22	21.5	*Escherichia coli, Aerobacter aerogenes,* and intermediates present.
5	35	34.4	None of the colon-aerogenes group present.

Groups 1 and 4 comprised for the most part shelling-plant samples. Groups 2 and 3 represent a comparatively even distribution with regard to origin of samples. Group 5 was principally made up of English walnut samples from domestic shelling plants.

SUMMARY

Nuts in the unbroken shell do not contain any members of the coliform group of bacteria.

Five hundred and forty-eight samples of nut meats, representing 11 varieties, were examined bacteriologically for evidence of *Escherichia coli*. Two hundred samples, purchased on the retail market, contained the organism in from four to 45 per cent of the samples. Two hundred and thirty-four domestic shelling-plant samples contained *Escherichia coli* in from six to 68 per cent. Examination of 114 import samples showed the presence of *Escherichia coli* in from 14 to 62 per cent.

Artificially contaminated nut meats stored at room temperature contained viable test bacteria *(Escherichia coli)* for approximately 68 days.

When large numbers of *Escherichia coli* are present, correspondingly large numbers of *Aerobacter aerogenes* and intermediates are found.

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REMOVAL OF LEAD IN MAPLE SYRUP BY MEANS OF BASE EXCHANGE MATERIAL[1]

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In an earlier paper Willits and Tressler (1938) discussed the sources of lead contamination of maple syrup and proposed a simple method for its removal. The present paper presents an improved method of eliminating lead either from diluted syrup or preferably from the sap itself.

The work of Adams (1930) indicated that the heavy metals could be removed from dilute solutions by percolating them through a zeolite, since it is known that substances will replace such metals as lead, zinc, copper, and tin when in solution with alkali or alkaline bases. His experiments dealt with the removal of these metals in concentrations of 100 p.p.m., while we are dealing with solutions (maple sap) containing only .01 to 1 p.p.m. of lead. However, it was thought that the process might be applicable to the removal of lead in maple sap.

The base-exchange material chosen for these investigations is "Zeo-Karb" — according to Tiger (1938) — a blackish granular material, which can be prepared by any one of a number of treatments of various carbonaceous materials, such as coal, lignite, or wood. The raw material, properly graded, is treated for extended periods with any one of a variety of chemicals, such as fuming sulphuric acid, sulphur trioxide, chlorosulphonic acid, or zinc chloride; after which it is washed, stabilized as required, and finally screened to the desired grain size. Whether or not the raw material has any exchange power at all, this drastic chemical treatment imparts to it a substantially increased ion-exchange power. Because of this and in accordance with modern usage of the term, this material is classed as a zeolite, and because of its carbonaceous origin, as a carbonaceous zeolite. Since this is a carbonaceous zeolite, it will not contribute silica to the percolating liquid, as would the other forms of zeolites; and because of its high capacity, it will require regenerating less often.

Zeo-Karb can function in the usual sodium-zeolite cycle, according to a communication from the Permutit Company, when regenerated with sodium chloride; or in the calcium-zeolite cycle, when regenerated with calcium chloride. Unlike all other zeolites, it can function

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in what may be called the hydrogen cycle, when regenerated with a strong acid such as sulphuric or hydrochloric.

The identification of pure maple syrup or the detection of its adulteration is most quickly done by the ash determination. This consists of determining the percentage of total ash and the ratio of the soluble to the insoluble ash as well as the ratios of their alkalinities, or the conductivity value of a syrup, which is a function of the ash content.

A base for the Zeo-Karb which will not alter the above analytical constants must be chosen. If the hydrogen Zeo-Karb is used the cycle is as follows:

$$\begin{matrix} Ca \\ Mg \\ K_2 \\ Na_2 \end{matrix} \text{ salts} + HZ \longrightarrow \begin{matrix} Ca \\ Mg \\ K_2 \\ Na_2 \end{matrix} Z + \text{acids of the salts}$$

and

$$\text{Pb salts} + HZ \longrightarrow PbZ + \text{acids of the salts.}$$

While this removes the foreign metal, the ash content of the syrup is also reduced to zero or well below the normal value.

If the sodium Zeo-Karb is used, the total alkalinity of the effluent is increased and the insoluble ash and its alkalinity are greatly reduced, thus making the ash determination ineffective as a means of detection of adulteration of the syrup.

When calcium Zeo-Karb is used, there is a base exchange between the calcium and the lead.

$$\text{Pb salts} + CaZ \longrightarrow PbZ + \text{Ca salts.}$$

According to Günther-Schulze's (1921) observations, the higher the atomic weight of a base, the more strongly it enters the permutite or zeolite. Thus even when the ratio of lead to sodium was 1:1000, the exchange process proceeded satisfactorily. The only important changes then, that are to be expected in the syrup prepared from the sap which has been deleaded by passing it through a calcium Zeo-Karb, would be an almost complete removal of the lead and a slight increase in the concentration of calcium. This increase in calcium will never exceed the limits of error of the method of the ash determination. For example, if a syrup contains 30 p.p.m. of lead, which is about the maximum concentration of lead ever found in maple syrup, then in a 10-gram sample taken for analysis, there will be .00003 gram of lead or .0000014 mole. Since the base exchange is mole per mole, there will be .0000014 mole or .00006 gram of calcium added to the syrup. This weight of calcium is an amount which is well within the limits of accuracy of the maple syrup ash deter-

mination, and it does not increase the total salts present in the syrup enough to alter the conductivity value.

EXPERIMENTAL PROCEDURE

One hundred and thirty c.c. (220 gm.) of the dry hydrogen Zeo-Karb were placed in a vertical tube, 3.5 cm. in diameter, which contained water through which the Zeo-Karb settled, forming a loosely packed bed through which maple sap (lead solution) would percolate freely. The tube was equipped with a stopcock at the bottom to regulate the flow of wash water and maple sap through the Zeo-Karb. The hydrogen Zeo-Karb was changed to the calcium Zeo-Karb by draining off the water and then adding a 10-per cent calcium chloride solution. This solution was kept in contact with the zeolite for threee days and then drawn off and the zeolite washed free of chlorides with water. The amount of calcium taken up by the Zeo-Karb was determined by analysis of the calcium chloride solution before and after treatment of the zeolite. This was found to be 1.710 grams or .0426 mole of calcium. This amount of calcium, if completely replaced by lead, will remove 8.822 grams of lead from solution.

Since no maple sap was available at the time of these experiments, they were conducted using simulated sap, which was prepared by diluting a pure maple syrup of high lead content with water until the sugar concentration was reduced to two or three per cent. This simulated maple sap, after percolating through the calcium Zeo-Karb, was evaporated to the density of pure maple syrup. All of the analyses were made on this recovered syrup or on the original syrup. All lead concentrations are therefore given in terms of standard syrup and they are not in terms of the lead in the sap solutions.

DISCUSSION

In these experiments the studies were limited to the relationship between the temperature, the contact time (rate of flow), the lead concentrations of the percolating sap, the base exchange power of the calcium of the calcium Zeo-Karb, and the lead in the simulated maple sap. The syrup from which the sap was made contained 3.85 p.p.m. of lead. Higher concentrations of lead were obtained by adding to the maple sap lead as lead acetate.

From the results of these experiments (Table 1) it is quite apparent that the amount of base exchange that takes place between the lead in solution and the calcium of the Zeo-Karb is a function of the contact time. The longer the time the more lead there is removed. The relationship, however, is not linear. From a practical viewpoint it is desirable that the flow of solution through the calcium

Zeo-Karb be kept at as high a rate as possible. Samples 4, 5, and 6 indicate that a contact time of one minute will reduce the lead concentration of more than six p.p.m. to one or less p.p.m. A shorter contact time did not remove the lead as completely as did a slower rate of percolation, as shown by Samples 2 and 5. A longer contact time removes more lead as shown by sample 3; however, the small increase in the amount of lead removal does not warrant the increase in time. For the successive experiments the contact time of one min-

TABLE 1

Effect of Contact Time and Lead Concentrations Upon Base-Exchange Power of Calcium of Calcium Zeo-Karb for Lead in Dilute Solutions

	Sample No.					
	1	2	3	4	5	6
Simulated maple sap						
Rate of flow, c.c. per min..	125	250	70	125	540	125
Contact time in min.....	1.0	0.5	1.7	1.0	0.2	1.0
Temperature, degrees C.....	20	20	4	....	....	....
Volume in liters.....	30.0	30.0	26.5	34.0	34.0	34.0
Per cent sugar.....	2.7	2.7	2.6	2.5[1]	2.5[1]	2.5[1]
Mg. lead added.....	0	0	3.0	3.0	0	0
Volume of syrup in ml. used to prepare simulated maple sap.....	1,000	1,000	800	1,000	1,000	1,000
Mg. lead in original syrup.....	4.62	4.62	3.70	4.62	4.62	4.62
Total mg. of lead present.....	4.62	4.62	6.70	7.62	4.62	4.62
Total amount of lead present in p.p.m. on basis of syrup...	3.85	3.85	6.08	6.85	3.85	3.85
P.p.m. of lead in syrup, obtained by evaporation of sap after it had been percolated through calcium Zeo-Karb.....	0.68	1.30	0.31	1.05	1.31	0.55

[1] Approximate percentage.

ute between the lead maple-sap solution and the calcium Zeo-Karb was used.

The effect of temperature on the base-exchange rate was studied by keeping the lead concentration and the contact time constant, while varying the temperature of the solution in contact with the calcium Zeo-Karb. The temperatures chosen were from 5 to 60°C. (41 to 140°F.), which range includes the temperatures of any stored maple sap from which maple syrup is processed. These temperature effects are shown (Table 2).

From these experiments it is shown that when the contact time is kept more or less constant, the base exchange between the calcium

Zeo-Karb and the lead of the contact solution is almost independent of the temperature between 5 and 60°C. When the lead concentration of the sap, in terms of syrup, was increased to 36 p.p.m. (Sample 25) and the contact time kept at one minute, the amount of lead remaining after treatment was less than one p.p.m.

To determine whether or not the base-exchange action of the calcium Zeo-Karb with the lead in the maple sap alters the syrup ash analytical constants, ash analyses were made. Comparison of the ash-analysis values of syrup Samples 21, 22, 23, and 24 with those

TABLE 2
Effect of Temperature on Base-Exchange Rate of Calcium of Calcium Zeo-Karb for Lead in Dilute Solutions

	Sample No.				
	21	22	23	24	25
Simulated maple sap					
Rate of flow, c.c. per min.	125	125	125	125	125
Contact time in min.	1.0	1.0	1.0	1.0	1.0
Temperature, degrees C.	20.5	40.0	60.0	5.0	20.0
Volume in liters	21.0	21.0	21.0	21.0	21.0
Per cent sugar	2.2	2.2	2.3	2.2	2.3
Mg. lead added	1.5	3.0	3.0	3.0	16.15
Volume of syrup in c.c. used to prepare simulated maple sap	500	500	500	500	500
Mg. of lead in original syrup	2.31	2.31	2.31	2.31	2.31
Total mg. of lead present	3.81	5.31	5.31	5.31	18.46
Total amount of lead present in p.p.m. of syrup	6.85	9.85	9.85	9.85	36.15
P.p.m. of lead in syrup obtained by evaporation of sap after it had been percolated through calcium Zeo-Karb	0.86	0.89	0.74	0.84	0.93

of the original syrup, from which the simulated sap samples were prepared, will indicate if any change has taken place. The results are given (Table 3) together with the maximum, minimum, and average ash-analysis values of several hundred maple-syrup samples.

In no case are the ash-analysis values (total, insoluble, and soluble) of the syrups prepared from the treated sap different from those of the original syrup, nor are the alkalinities of the soluble and insoluble ash different from those of the original syrup.

SUMMARY

Calcium Zeo-Karb (zeolite) will effectively remove lead from maple sap even when the lead concentration is as much as 36 p.p.m. in terms of syrup. This base-exchange action of the calcium of the

TABLE 3

Effect of Base Exchange of Calcium for Lead in Maple Syrup on Ash Analysis of the Syrup, Calculated on Dry Basis

	Sample No.							
	Orig.	21	22	23	24	Average [1]	Maximum [1]	Minimum [1]
Ash analysis								
Total (per cent)	0.98	1.03	1.07	0.95	0.90	1.00	1.68	0.68
Soluble (per cent)	0.56	0.56	0.58	0.55	0.47	0.63	1.23	0.35
Insoluble (per cent)	0.43	0.43	0.45	0.39	0.44	0.37	1.01	0.23
Ratio soluble: insoluble ash	1.30	1.30	1.28	1.41	1.14	1.70	3.80	0.53
Alkalinity of								
Soluble ash (c.c. of .1 N acid per gram sample)	0.64	0.69	0.70	0.67	0.56	0.75	1.22	0.41
Insoluble ash (c.c. of .1 N acid per gram sample)	1.10	1.10	1.10	1.04	0.99	0.97	2.08	0.41
Ratio alkalinity soluble ash: alkalinity insoluble ash	0.58	0.63	0.64	0.64	0.57	0.77	1.83	0.21

[1] Results of analysis of several hundred maple-syrup samples, representing a summary of all the leading analyses of genuine syrups as given by Snell and Scott, "The Analysis of Maple Products. III." J. Ind. Eng. Chem. **6**, 216 (1914).

calcium Zeo-Karb and the lead in the maple sap are independent of the temperature in the range between 5 and 60°C.(41 and 140°F.), but it is dependent upon the contact time (rate of percolation of the sap through the zeolite). When a contact time of one minute was used, the lead concentration was effectively reduced from 36 p.p.m. to less than one p.p.m.

The present study has been limited to the removal of lead from maple sap by means of a base-exchange material; however, the authors are continuing this work and are now conducting experiments on the removal of zinc, copper, iron, and tin from maple sap with this material.

During the 1939 maple-sugar season a calcium Zeo-Karb treatment plant will be set up and used in a sugar bush to ascertain its value under actual operating conditions.

This paper is written before the completion of the work on the removal of the other heavy metals from maple sap, since the question of lead in maple products is very keen at the present time and the authors feel that this paper may be of some value to other workers.

The use of this base-exchange material would be extremely simple. It would only need to be assembled in the pipe line leading from the maple-sap storage tank to the evaporator. It should require no attention whatever except to recharge it with calcium chloride at the beginning of each sugar season. The Zeo-Karb, if kept clean and washed well after each season's use, should last indefinitely.

CONCLUSIONS

1. Calcium Zeo-Karb (zeolite) will remove lead from maple sap through base exchange.

2. Between the temperatures of 5 and 60°C. the amount of lead removed by the zeolite is independent of the temperature.

3. The amount of lead removed is dependent upon the contact time.

4. Using a contact time of one minute the lead concentrations in maple syrup can be reduced from 36 p.p.m. to one or less p.p.m.

5. Percolating maple sap through calcium Zeo-Karb does not in any way alter the ash values of the syrup prepared from this simulated sap.

ACKNOWLEDGMENT

The authors wish to express their appreciation to Mr. H. L. Tiger, vice-president of the Permutit Company, New York City, for his valuable assistance.

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SIMULATION, BY CHEMICAL AGENTS, OF COOKING OF POTATO TISSUE

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Two obvious changes occur in potato tissue during cooking: the starch is gelatinized and, owing to a marked decrease in cell adhesion, the tissue is softened so that it can be readily mashed. If these two alterations are those prerequisite to the conversion of raw tissue into a product having the physical qualities associated with cooked potato, it should be possible to obtain the same results by chemical means without the agency of heat. This was accomplished by treating slices of potato first, with chemicals that would bring about cold gelatinization of the starch, and subsequently, with oxalates which are known to be effective in decreasing the cell adhesion of plant tissue.

EXPERIMENTAL PROCEDURE

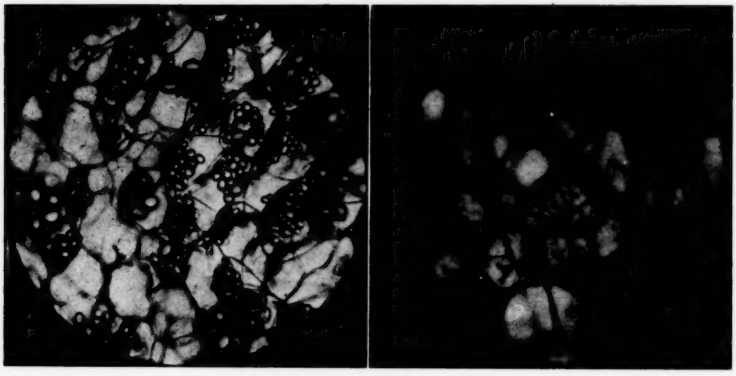
Slices of potato tuber were immersed in a number of solutions which produced cold gelatinization of the starch. Microscopic observation of the treated tissue showed that for the most part the individual starch grains had swollen sufficiently to be indistinguishable and to completely fill the tissue cells (Fig. 1, B). This caused no reduction in the tensile strength of the tissue, clearly indicating that gelatinization per se does not produce doneness and is not the specific alteration which transforms raw potato into a condition satisfactory for mashing. The following reagents produced cold gelatinization of potato tissue:

Chloral hydrate—40 per cent solution, at room temperature in about 48 hours.

Formaldehyde—40 per cent solution, at room temperature in 5 to 6 days.

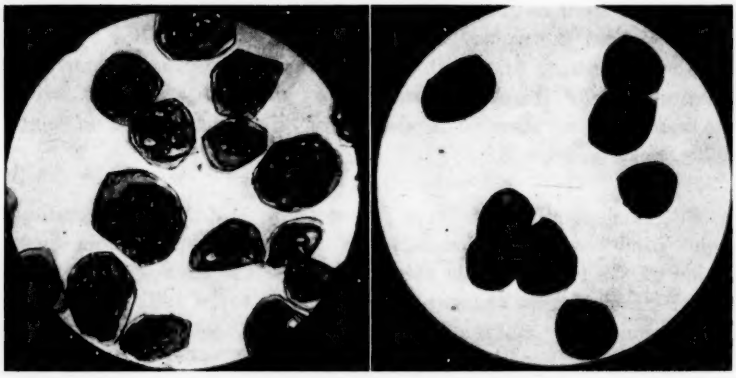
Sodium salicylate—2 N solution, at room temperature in about 48 hours.

Ammonium thiocyanate—solution containing 40 grams ammonium thiocyanate, 200 ml. water and 50 ml. 95 per cent ethyl alcohol, at 40 to 50°C. (104 to 122°F.) in 20 to 40 hours.



A
Untreated raw potato tissue

B
Chloral hydrate treated potato tissue



C
Ammonium oxalate treated potato tissue

D
Chloral hydrate and ammonium oxalate treated potato tissue

FIG. 1. Photomicrographs showing the effect of various chemical treatments on raw potato tissue. (Magnification approximately 50X.)

A. Untreated raw potato tissue.

B. Potato tissue after 48 hours in 40 per cent chloral hydrate at room temperature.

C. Potato tissue after 25 hours in .5 per cent ammonium oxalate at 65°C.(149°F.).

D. Potato tissue after 48 hours in 40 per cent chloral hydrate at room temperature and 25 hours in .5 per cent ammonium oxalate at 65°C. (149°F.).

Previous work had demonstrated that softening, like that ordinarily obtained during cooking, can be accomplished by holding potato tissue for about 25 hours in a .5 per cent solution of ammonium oxalate at 65°C.(149°F.), Personius and Sharp (1939). Presumably the oxalate weakens the intercellular cementing material of the tissue by removing calcium from the calcium pectate of the middle lamella. Observed under the microscope, oxalate-treated tissue was found to have separated quite completely into individual cells. The starch grains had swollen somewhat, but still an appreciable volume of each cell was unoccupied by starch (Fig. 1, C). Although this product could be mashed easily, it did not look like cooked potato. Therefore, doneness of potato tissue, so far as ease of mashing is concerned, is associated with a marked decrease in cell adhesion but this alteration alone does not produce cooked tissue.

When cold gelatinization of the starch and oxalate treatment to decrease cell adhesion were carried out on the same piece of potato, the tissue was so altered that it mashed readily and in all outward appearances closely resembled cooked potato. The flavor, however, was still that of raw potato. Photomicrographs of this material, like those of cooked tissue, show many separated cells each completely filled with swollen or gelatinized starch grains (Fig. 1, D).

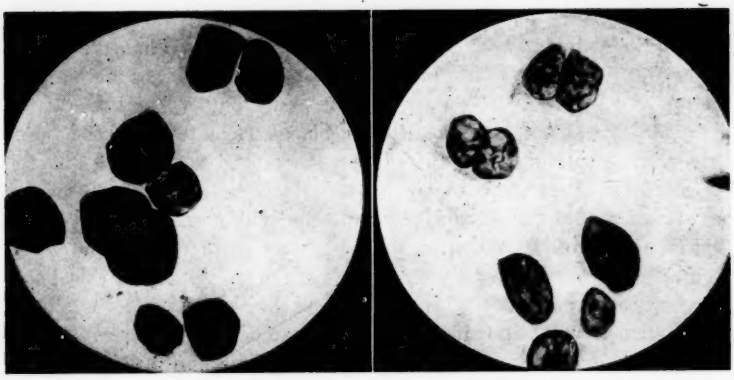
A previous paper reported that after having been decreased by means of ammonium oxalate, the cell adhesion of potato tissue can subsequently be increased by treatment with solutions of calcium chloride, Personius and Sharp (1939). Similar treatment of "chemically cooked" tissue, which has acquired the property of easy mashing, causes the tissue to revert to a firm physical state that resists mashing to the same extent as underdone potato tissue.

A large number of potato slices have been "cooked" by the chemical process, chloral hydrate or ammonium thiocyanate being used as the gelatinizing agent in most cases. It was observed that tubers from a lot yielding soggy potatoes when steamed or boiled, also yielded soggy products when "chemically" cooked. Similarly, mealy potatoes were alike in texture when cooked by heat and when cooking was simulated by chemical means.

Representative photomicrographs of soggy and mealy potatoes are shown (Fig. 2). Each set was obtained from one tuber, one half of which was cooked by steaming while the other half was treated with appropriate solutions of chloral hydrate and ammonium oxalate.

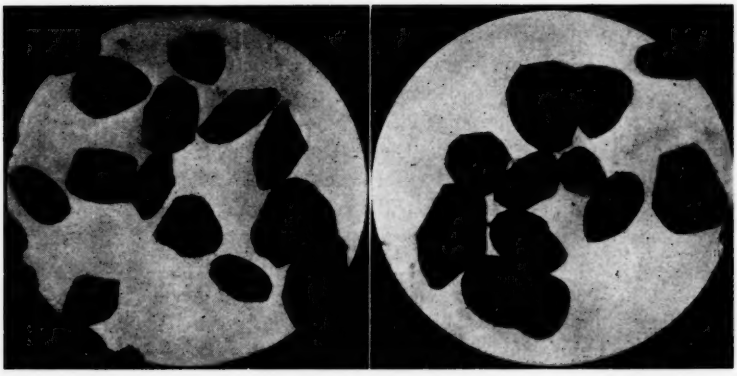
SUMMARY

The starch of potato tissue can be gelatinized by any one of a number of cold gelatinizing agents. By treatment with ammonium



A
Soggy potato cooked

B
Soggy potato chemically treated



C
Mealy potato cooked

D
Mealy potato chemically treated

FIG. 2. Photomicrographs showing soggy and mealy potato tissue (1) after steaming to an internal temperature of 98°C.(208°F.) and (2) after holding for 48 hours in 40 per cent chloral hydrate at room temperature, then for 25 hours in .5 per cent ammonium oxalate at 65°C.(149°F.). Each set of pictures was obtained from one tuber, one half of which was cooked by heat while the other half was treated with appropriate chemical solutions. (Magnification approximately 50X.)

oxalate the adhesion between the cells of potato tissue can be reduced to that characteristic of cooked tissue. It is possible to simulate cooking by first treating potato tissue with a cold gelatinizing agent and subsequently with ammonium oxalate. The texture of potato tissue appears to be the same whether cooking is simulated by chemical means or is accomplished by heat.

It appears that two fundamental changes are necessary for converting raw potato into cooked tissue: (1) gelatinization of the starch and (2) a marked decrease in the cell adhesion of the tissue. Neither of these alterations, alone or in combination, offers a satisfactory explanation for the differences between so-called mealy and soggy potatoes. Some other factor must be involved in determining the texture of potatoes.

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CAROTENE (VITAMIN A) CONTENT OF FRESH AND FROSTED PEAS[1,2]

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On account of the rapid growth of the frosted-vegetable industry, the effect of blanching and other processes of preparation for freezing and storage on the vitamin content of vegetables is of considerable importance. The effect of these commercial processes on the vitamin C content of frozen vegetables has been considered by the following investigators: Fellers, Young, Isham, and Clague (1934); Fellers and Stepat (1936); Fellers, Stepat, and Fitzgerald (1936); Dunker, Fellers, and Fitzgerald (1937); Tressler, Mack, Jenkins, and King (1937); Jenkins, Tressler, and Fitzgerald (1938a); Fenton and Tressler (1938). Their work has shown that in general, freezing in itself does not cause a loss of vitamin C. On the other hand, blanching and other processes employed in preparing vegetables for freezing cause some loss of this vitamin. This loss varies from an almost negligible amount in the case of sweet corn to approximately 30 per cent in peas and even 35 or 40 per cent in spinach.

Jenkins, Tressler, and Fitzgerald (1938b) have recently shown that unless frozen peas and other vegetables are stored at very low temperatures—for example, —40°C.(—40°F.)—vitamin C is slowly lost during cold storage.

Using the bioassay method, Fellers, Young, Isham, and Clague (1934) found that frozen asparagus contained as much vitamin A as the fresh product. From this, one may conclude that no loss is caused either by preparation for freezing or by freezing of asparagus.

Fitzgerald and Fellers (1938) determined the carotene content of 15 frozen vegetables and compared these results with the vitamin A values for the fresh vegetables reported in the literature. They conclude: "It is apparent that freezing causes no measurable loss in vitamin A activity (carotene) of vegetables, if they are kept in a solidly frozen condition." On the basis of this work, this conclusion does not seem entirely warranted as Fitzgerald and Fellers did not

[1] Approved by the director of the New York State Agricultural Experiment Station for publication as Journal Paper No. 312, February 3, 1939.

[2] Based principally upon a thesis by Clinton R. Stimson and presented to the faculty of Cornell University in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

prove that the carotene determined by them was all beta-carotene nor did they show that their modification of the Russell, Taylor, and Chichester (1935) method did not include in the extract pigments other than beta-carotene. Further, factors such as variety, maturity, and freshness of the vegetables were not considered.

Employing both the bioassay and chemical methods, DeFelice and Fellers (1938) compared the carotene content of fresh and frozen spinach and observed that the frozen product contained about three fourths as much as the fresh vegetable on the moist basis. They did not show whether the loss occurred prior to or after freezing.

The present study was undertaken to determine the following points: (1) the reliability and accuracy of chemical methods when applied to the determination of carotene in peas, (2) whether or not there is any appreciable loss of carotene during the commercial preparation of peas for freezing, (3) if there is a loss of carotene from peas during freezing and subsequent cold storage.

PREPARATION OF PEAS

The peas used in this study were of the Thomas Laxton variety obtained from the Albion, New York, plant of the Snider Packing Corporation. In order to eliminate as far as possible the effect of maturity on the carotene content of the vegetable, part of the peas from one load were, after vining, graded (sieved) so as to eliminate all except size No. 6. In this way a large uniform lot of peas was obtained. These graded peas were prepared for freezing and were frozen according to the usual procedure followed in that particular plant. This procedure involves the following steps: (1) washing in an Olney washer, (2) scalding in a continuous water blancher at 98.9°C.(210°F.) for 40 seconds, (3) cooling first with sprays of cold water and then in running water which conveys them through a flume to a brine "quality grader," (4) separation by brine of the overmature peas (quality grading), (5) washing to eliminate brine, (6) conveying by water in a flume to (7) an inspection belt where any foreign matter and yellow peas are eliminated, (8) draining, (9) filling into moisture-proof, cellophane-lined, Peters-type cartons, (10) wrapping the cartons with a heat-sealing, moisture-proof wrapper, (11) freezing between metal plates at approximately —31.7°C. (—25°F.) in a Birdseye multiplate froster.

As the peas passed through the plant, samples were taken for analyses at the following points: (a) before vining, (b) after vining, (c) after blanching, cooling, quality grading, inspecting, and packaging, (d) after quick freezing. The unfrozen samples were analyzed as soon as possible for carotene, total solids, and alcohol-

insoluble solids. Some of the frozen samples were analyzed immediately; the remainder was divided into two lots, one stored at —17.8°C. (0°F.) and the other at —40°C.(—40°F.).

The remainder of the load of peas was not graded for size but was put through the regular commercial procedure of preparation and freezing. These peas, which are listed in the tables as the commercial sample, were stored at 0°F.

ANALYTICAL METHODS

Carotene determinations were run in triplicate, by each of two methods, on the fresh peas and in duplicate by these two methods on the freshly frozen peas. One of the methods used was the Russell, Taylor, and Chichester (1935) modification of the Willstätter and Stoll's (1913) acetone-extraction procedure. The other was the Peterson, Hughes, and Freeman (1937) modification of the alcoholic-potash procedure of Guilbert (1934).

Alcohol-insoluble solids were determined by the method described by Kertesz (1935a, b). Total solids were also determined. The latter determinations were run to obtain an index of the maturity of the samples and to permit calculation of results on a dry-matter basis.

Carotene and total-solids determinations were repeated after five, nine, and 11 months' storage in order to determine whether or not carotene is lost during storage of frozen peas. Since the results of determinations on the fresh and freshly frozen peas seemed to indicate that the acetone-extraction procedure is more reliable than the Guilbert alcoholic-potash procedure, the former was the only one used on the stored samples.

BIOASSAY METHOD

In order to check on the accuracy of the Russell, Taylor, and Chichester method, a bioassay of the stored peas was carried out. The assay was conducted as nearly as possible according to the official method outlined in the *United States Pharmacopoeia XI* (1935). Approximately 10 rats were placed in all groups except the positive-control and negative-control groups, which contained only five and six rats, respectively. A few rats were used in the assay, because of a shortage of animals, which did not meet official specifications. In no case was there any justification for excluding the response of these animals from the final data.

The amount of peas fed in the biological assay was determined on the basis of the December carotene determinations. Levels of peas containing .6, 1.2, and 1.8 gamma of carotene per day per rat were fed twice weekly during the assay period for each of the three samples of peas stored. A negative-control group receiving only the

basal diet and a positive-control group receiving three units of standard reference cod-liver oil daily were fed. Also two reference groups were reared, one receiving .6 gamma of pure beta-carotene daily and the other 1.4 units of reference oil daily. These groups receiving oils and beta-carotene as supplements received these supplements diluted to .1 c.c. with Wesson oil daily.

DISCUSSION OF RESULTS

From the data given on the content of carotene, alcohol-insoluble, and total solids of the fresh and freshly frozen peas (Table 1) it is apparent that the vining, blanching, washing, and other processes incidental to preparation for freezing cause the loss of little or no

TABLE 1

Changes in Carotene Content of Thomas Laxton Peas During Preparation and Freezing

	Before vining [1]	After vining [1]	After Blanching [1]	After freezing	
				No. 6 size [2]	Commercial
Total solids, per cent	23.0	24.2	24.5	24.5	20.1
Alcohol-insoluble solids, per cent	18.5	20.1	19.5	19.2	12.7
Gamma of carotene per gram fresh sample (acetone method)	5.2	4.9	5.9	4.5	4.7
Gamma of carotene per gram dry weight (acetone method)	22.6	20.2	23.8	18.4	23.3
Gamma of carotene per gram fresh sample (alcohol-KOH method)	4.6	4.0	3.9	4.8	4.9
Gamma of carotene per gram dry weight (alcohol-KOH method)	19.9	16.5	15.8	19.5	24.2

[1] Graded to size No. 6. [2] Average of analyses of the two parts of graded sample as shown in Table 2.

carotene. The Russell, Taylor, and Chichester method gives results somewhat higher, on the average, than the Guilbert method. The latter method appears to give much more variable results than the former.

The ungraded (commercial) samples were somewhat higher in carotene than those graded to size No. 6; it is probable that this is because, on the average, the former contained less mature peas. The total solids and alcohol-insoluble solids (indices of maturity) are much lower in the case of the ungraded sample.

The results of the analyses made to determine changes in carotene content during storage of the frozen product (Table 2) show that at —40°C.(—40°F.) no loss of carotene occurs in 11 months' storage. On the other hand, at —17.8°C.(0°F.) both samples showed a defi-

nite loss of carotene, viz., seven per cent for the graded sample and 26 per cent for the ungraded sample, an average of approximately 16 per cent. These results are similar to the losses of vitamin C from frosted peas at 0°F., observed by Jenkins, Tressler, and Fitzgerald (1938b).

After storage for five months carotene determinations on the frosted peas were made in preparation for the bioassays. On the basis of these analyses the amounts of peas to be fed were calculated. Later it was found that these results were approximately 15 per cent too low.[3] On account of this error the rats fed on the lowest level of peas received not more than 15 per cent more carotene than did

TABLE 2

Carotene Content of Frosted Peas After Storage

Component	Storage period	Graded (Size 6)		Ungraded (commercial)
		Stored at —17.8°C. (0°F.)	Stored at —40°C. (—40°F.)	Stored at 0°F.
	mo.			
Total solids, per cent	0	24.6	24.5	20.1
	9	24.6	24.0	20.3
	11	22.3	24.2	23.6
Gamma of carotene per gram fresh sample	0	4.6	4.4	4.7
	9	4.0	4.4	3.8
	11	3.9	4.5	4.1
Gamma of carotene per gram dry matter	0	18.7	18.1	23.3
	9	16.1	18.5	18.7
	11	17.5	18.6	17.2

those fed on pure beta-carotene. This condition was reflected in the slightly better average weekly gain of the rats fed peas when compared with those fed beta-carotene (Table 3). However, in general, it is evident that entirely similar responses were obtained for all samples of peas fed at the .6 gamma level and also the .6 gamma level of pure beta-carotene. Statistical analysis of these data shows no significant difference between the average weekly gains of any groups fed on the .6 gamma levels, and none between the groups fed on the 1.2 and 1.8 gamma levels, respectively.

Comparing the graded sample stored at 0°F. with the pure carotene, there is a ratio between the average weekly gains of 1.26; while

[3] In the case of the commercial sample the apparent error is much less than 15 per cent, probably because the commercial peas were not nearly as uniform in maturity as the samples graded to size No. 6.

TABLE 3

Bioassay of Frozen Peas

Sample	Amount of carotene per gram fresh sample		Amount of carotene fed daily		Amount peas fed daily per rat	Amount peas fed per period per rat	Average weight of rats		Average weekly gains $\pm \sigma M$
	Based on analyses of 12/29/37	Based on average analyses of 5/3 and 7/1/38	Based on analyses of 12/29/37	Based on average analyses of 5/3 and 7/1/38			At start	At termination	
	gamma	*gamma*	*gamma*	*gamma*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Graded,[1] stored at —17.8°C.(0°F.)	3.5	3.9	0.6	0.67	.170	4.75	104.2	150.6	11.59 ± 1.06
			1.2	1.33	.339	9.49	114.2	174.1	14.97 ± 2.10
			1.8	2.00	.509	14.24	104.0	175.2	17.81 ± 1.84
Graded,[1] stored at —40°C.(—40°F.)	3.9	4.5	0.6	0.69	.155	4.34	121.1	160.3	9.80 ± 1.11
			1.2	1.39	.310	8.68	118.6	169.9	12.83 ± 1.82
			1.8	2.08	.465	13.02	131.5	194.5	15.75 ± 1.48
Commercial stored[1] at 0°F.	3.7	3.9	0.6	0.64	.162	4.54	110.3	149.0	9.68 ± 1.73
			1.2	1.27	.324	9.08	123.2	182.9	14.92 ± 1.67
			1.8	1.91	.487	13.62	130.5	192.9	15.60 ± 1.29
	Amount fed daily								
Beta-carotene	0.6 gamma						138.6	175.4	9.19 ± 1.26
Reference oil	1.4 units						123.7	152.8	7.28 ± 1.12
	3.0 units						113.2	174.8	15.40 ± 3.18

[1] Storage period was from six to eight months.

with the graded sample stored at —40°F. and the commercial sample, there are ratios of 1.07 and 1.05, respectively. Statistical analysis indicates that this slight variation is probably due entirely to chance.

The positive-control group receiving three units of reference oil daily made gains which agree well with those of the groups receiving peas in amounts containing 1.8 gamma of carotene daily. Thus one unit of reference oil seems equal to .6 gamma of carotene at this feeding level. At lower levels, the 1.4 units of reference oil did not produce as much growth as the .6 gamma levels of carotene in peas and of beta-carotene. Statistically, however, this difference is not significant and is probably entirely due to chance. The positive-control group receiving three units of reference oil daily made gains 2.12 times that of the 1.4 units reference-oil group, and they were receiving 2.14 times as much vitamin A as supplement. Larger growth responses were obtained in this experiment than would be expected from the reports of other assays, but these responses are in accord with the results of other workers in our laboratory.

The graded sample of peas studied had 17.2 gamma of carotene per gram of dry matter, while the commercial sample had 19.3 gamma of carotene per gram of dry matter. Since .6 gamma of carotene is equivalent to one International Unit of vitamin A, these samples would have 28.7 I.U.[4] and 32.2 I.U. per gram of dry matter, respectively. When the *United States Pharmacopoeia XI* formula [5] is used to calculate the vitamin A content, values of 25.4 I.U. and 29.1 I.U. per gram of dry matter, respectively, for these two samples were obtained. Close agreement is obtained by these two different methods of calculating the vitamin A content. These samples contained 6.1 and 6.2 I.U. of vitamin A, respectively, on a fresh-weight basis.

The ratio of one unit of vitamin A to .6 gamma of carotene is 1.67. The ratio of vitamin A to carotene was 1.48 in the graded peas and 1.51 in the commercial peas, which values agree well with the theoretical value of 1.67 and that observed by DeFelice and Fellers (1938) and Fraps, Treichler, and Kemmerer (1936) on other plant materials. Here again is evidence that the carotene in peas has an equivalent biological value to beta-carotene.

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[4] I.U. = International Units.

[5] $\frac{R}{A}$ x I.U. per gram of reference oil = I.U. per gram of supplement fed.

R equals the daily dose in milligrams of the reference oil necessary to produce in a reference-oil group an average gain in weight of not less than 12 and not more than 60 grams. A equals the daily feeding in milligrams of the supplement that will produce in an assay group an average gain in weight equal to or greater than that produced in the reference-oil group.

SUMMARY AND CONCLUSIONS

The carotene content of freshly frozen, blanched Thomas Laxton peas is substantially the same as that of the freshly harvested vegetable. During storage for 11 months at —40°C.(—40°F.) there is no appreciable loss of carotene, but at —17.8°C.(0°F.), on the other hand, a loss of from seven to 26 per cent apparently occurs during this period.

The international unitages of vitamin A in peas, calculated both from the carotene content and from the bioassay, agree well, viz., about 6.1 I.U. on fresh basis. The ratio of vitamin A to carotene averaged 1.5 which checks closely with the theoretical value of 1.67.

Bioassays indicated that the Russell, Taylor, and Chichester (1935) modification of the Willstätter and Stoll (1913) method of determining carotene gives satisfactory results in the case of peas. The method is sensitive and accurate. Since the bioassay and chemical methods check so closely, all of the carotene extracted from peas appears to have the same vitamin A potency as beta-carotene.

ACKNOWLEDGMENT

The authors wish to acknowledge the aid of W. I. Zimmerman, who carried out some of the chemical determinations reported in this paper.

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SUSCEPTIBILITY OF FROZEN-DEFROSTED POULTRY MEAT TO DRIP

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A conventional method used in determining the effects of freezing on meat products has been to study, principally quantitatively, the drip associated with various freezing conditions. Numerous papers have appeared dealing with the effects of freezing on beef and other meats, but little attention has been given to poultry. Recently, however, Sair and Cook (1938) have studied the effects of the rate of freezing and other factors as related to drip in poultry. These authors have concluded, after making quantitative studies on drip on frozen-defrosted whole birds, that regardless of rate of freezing, whole birds do not drip but that removal of skin, cutting the meat, and particularly mincing the meat increase the susceptibility of the frozen-defrosted tissues to drip.

It is a matter of considerable interest to determine whether or not frozen-defrosted poultry meat has the capacity to retain reabsorbed water, particularly when the product is cooked, and one of the objectives of the present study has been an investigation relating to this point. Of primary concern, however, have been studies regarding the susceptibility of dark and white muscle to drip as related to freezing temperatures. The relationship of drip to the time of freezing after slaughter was also studied.

COOKING SHRINKAGES AS RELATED TO FREEZING TEMPERATURES

In an attempt to ascertain the cooking shrinkage identified with various freezing temperatures, 20 birds were frozen at —45.5°C. (—50°F.), —26.1°C.(—15°F.), and —13.3°C.(8°F.), and a similar number of unfrozen birds were used as controls. The birds were laboratory raised and only those were selected for the test that showed considerable uniformity as regards age, size, condition, and body conformation. They were held for 24 hours at 3.3°C.(38°F.) before being drawn. The average weight of the drawn birds was 995 grams. The unfrozen or control birds were cooked immediately after being drawn. The others were frozen in air, ammonia being used as the refrigerant to cool the air, except in the case of those frozen at —50°F., for which dry ice was used as the refrigerant.

The birds remained in the freezer for a period not exceeding four days, after which time they were removed to a 38°F. cooler to defrost. After defrosting for three days the birds, with giblets contained in body cavity, were roasted in similar ovens heated to 162.8°C.(325°F.) and were considered cooked upon attaining an internal temperature of 90.6°C.(195°F.). Five birds were simultaneously roasted in an oven and individual weights were recorded for each bird.

The results of the cooking tests (Table 1) indicate that the differences in average cooking shrinkages between the unfrozen birds and those frozen at the several temperatures were not significant.

TABLE 1

Average Cooking Shrinkages in Frozen and Unfrozen Birds

Identification of birds	Number of birds	Cooking shrinkage	Standard deviation
		pct.	
Unfrozen	20	26.3	3.36
Frozen, —45.5°C.(—50°F.)	20	25.2	3.09
Frozen, —26.1°C.(—15°F.)	20	26.9	3.33
Frozen, —13.3°C.(8°F.)	20	25.6	3.42

There were, however, rather large differences in shrinkage between individual birds.

HISTOLOGY OF FROZEN AND DEFROSTED MUSCLE FROM REPRESENTATIVE BIRDS

Histological preparations were made of muscle tissues from representative frozen birds in accordance with the method recently suggested by Koonz and Ramsbottom (1939). It was found that intrafiber freezing occurred in birds frozen at —45.5°C.(—50°F.), whereas extrafiber freezing occurred in the birds frozen at —13.3°C.(8°F.). In the birds frozen at —26.1°C.(—15°F.) extrafiber freezing occurred; however, the fibers were not forced into ridge-like formations owing to the large ice crystals, as in the case of more slowly frozen birds, but remained rather uniformly dispersed and distributed.

Histological preparations were also made of defrosted muscle tissue and, irrespective of freezing temperatures, the fibers show remarkable capacities to reabsorb or appropriate the "frozen out" or displaced water. This becomes apparent upon examining dark, striated muscle tissue from slowly frozen birds (Fig. 1) and comparing it with similarly frozen-defrosted tissue (Fig. 2). It will be noted that in the slowly frozen tissue water has been lost by the

fibers during freezing and that it has been frozen outside of the fibers, forming large extrafiber ice crystals. Upon being defrosted, however, the water has been reabsorbed by the muscle fibers and the tissue has become quite normal in appearance. This is likewise true for the defrosted muscle tissue from birds previously frozen at —15°F. and —50°F.

DEFROSTING LOSSES IN GROUND DARK AND WHITE POULTRY MEAT

It is generally recognized that frozen-defrosted whole birds do not drip materially. Therefore, in order to study the susceptibility of the dark and white meat to drip, the meat was ground. Defrosting shrinkage was ascertained for ground dark and white meat frozen at —50°F. and at 8°F. The susceptibility of the frozen-defrosted poultry meat to drip was studied for muscle tissue from birds pre-

FIG. 1. Poultry muscle frozen at —13.3°C.(8°F.), X83.

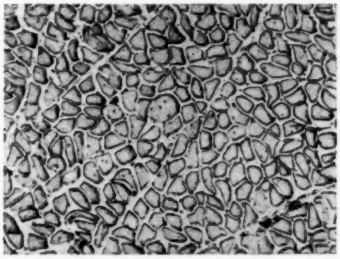

FIG. 2. Thawed poultry muscle previously frozen at —13.3°C.(8°F.), X83.

cooled for 24 hours. Likewise, drip was determined for muscle tissue frozen between two and three hours after slaughter of birds.

The birds from which the muscle tissue was taken weighed approximately 1,250 grams before being drawn. After the birds were defeathered no water was applied to the tissues. Seventy grams of ground muscle were placed into 100-ml. beakers and formed into small molds. The molds were removed from the beakers and remained intact during subsequent treatment. Only two molds were made from each bird, one consisting of dark muscle and the other of white muscle. The molds were held in the frozen condition for three days, after which time they were placed in large closed containers, on raised wire meshings, and permitted to defrost for two days at 10°C. (50°F.)

Drip arbitrarily may be considered as the loss occurring during the two days, based on weights of the molds taken immediately be-

TABLE 2

Comparison of Defrosting Losses for Ground Dark and White Poultry Meat

	Test	White meat				Dark meat			
Precooling temperature, °F	A, B	....	....	38	38	....	....	38	38
Time frozen after slaughter, hr	A, B	2-3	2-3	24	24	2-3	2-3	24	24
Freezing temperature, °F	A, B	8	—50	8	—50	8	—50	8	—50
Number of molds	A	7	7	7	7	7	7	7	7
Average defrosting shrinkage, per cent	A	6.90	6.80	9.87	6.80	2.90	2.47	2.71	2.20
Standard deviation	A	0.63	0.63	1.32	0.63	0.00	0.67	0.92	0.69
Number of molds	B	6	6	6	6	6	6	6	6
Average defrosting shrinkage, per cent	B	3.06	3.40	3.00	1.33	1.73	1.63	0.00	0.00
Standard deviation	B	1.71	0.49	0.75	0.67	0.33	0.33	0.00	0.00

fore and after the defrosting period. This is not an absolutely accurate estimation of drip, for it is obvious that part of the loss may be due to evaporation.

Two independent tests were made in an attempt to ascertain defrosting losses for the dark and white muscle tissue; results of the tests are summarized (Table 2). For some unknown reason, the defrosting losses are considerably larger in Test A than in Test B; however, similar trends exist in both tests.

It will be noted that the shrinkages occurring during the two-day defrosting period were considerably larger for the white meat than for the dark meat. A part of the shrinkage identified with the dark meat may be attributed to evaporation losses. It becomes evident that the ground dark meat, at most, exhibits only a very slight susceptibility to drip. The white poultry meat showed a definite susceptibility to drip, which appeared to be independent of freezing temperatures when frozen within three hours after slaughter of birds. In the molds composed of muscle tissue taken from birds precooled for 24 hours previous to freezing, the average defrosting loss was greater at —13.3°C.(8°F.) than at —45.5°C.(—50°F.) and follows the rather conventional relation between quantity of drip and freezing temperature.

Marked differences were found to exist in the hydrogen-ion concentrations of the dark and white muscle. The pH determinations were made on ground muscle, to which equal amounts of distilled water had been added, employing a Beckman pH meter with glass electrode and calomel electrode. Each pH value (Fig. 3) represents an average of determinations made on ten birds. The birds were held at 3.3°C.(38°F.) and the same birds were used throughout the test. The birds were of similar size, the average weight being 1,147 grams after being defeathered.

The pH values taken between two and three hours after slaughter of birds were 5.7 for white meat and 6.1 for dark meat. From these pH values and from the values secured on subsequent days (Fig. 3), it will be noted that the dark muscle has a higher pH than white muscle. The white muscle with the lower pH values does not reabsorb the "frozen out" water as readily as the dark muscle with the higher pH values. Whether this lack of the reabsorptive capacity exhibited by the white muscle is due to the lower pH values or whether the relationship is merely incidental has not been determined.

Moisture determinations were also made on the same birds from which pH values were secured. These determinations were made one day after slaughter of birds. The average for ten birds showed that

the white muscle contained 74.6 per cent water, whereas the dark muscle contained 73.3 per cent water.

DISCUSSION

The results of the present test on free drip indicate that the precooled ground white muscle drips more when frozen at —13.3°C. (8°F.) than when frozen at —45.5°C.(—50°F.). Koonz and Ramsbottom (1939) have shown that when poultry muscle tissue is rapidly frozen, small intrafiber ice columns occur; and when slowly frozen, larger, but fewer, extrafiber ice crystals occur. The size, distribution, and number of ice formations apparently influences

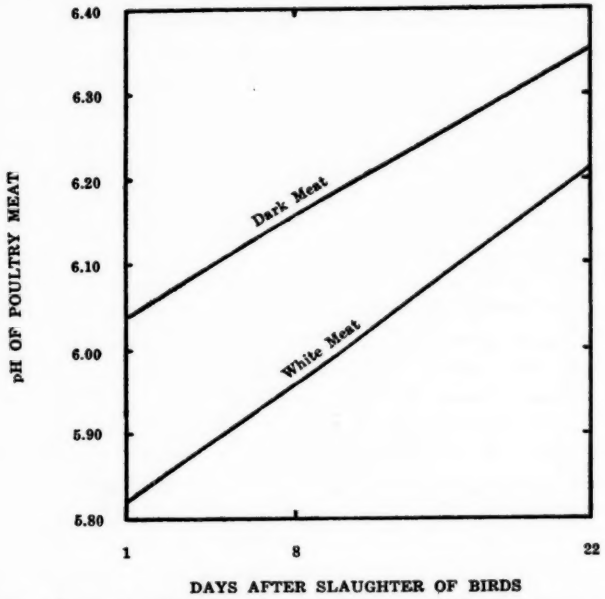

FIG. 3. Comparison of pH values for dark and white poultry meat. Each pH value represents an average of ten birds. The birds were held at 3.3°C.(38°F.) during the 22-day period that the pH observations were being made.

the production of free drip in the precooled ground white muscle. In the more rapidly frozen muscle tissue the water is more accessible to the partially dehydrated fibers than in the slowly frozen tissue. A large fiber-water surface relation will facilitate reabsorption and will minimize drip.

Sair and Cook (1938) have pointed out that regardless of the rate of freezing, whole birds do not drip. In the present study a

histological study of frozen-defrosted muscle tissue from whole birds showed that the fibers, irrespective of freezing temperature, exhibited remarkable capacities for reabsorbing the "frozen out" water. Even slowly frozen muscle tissue (Fig. 1) containing large extrafiber ice crystals when defrosted (Fig. 2) appears to reconstitute very satisfactorily. In whole birds the area of cut surface to volume of meat is extremely small and the muscle fibers have opportunity to completely reabsorb the "frozen out" water. Cooking data indicated that the muscle fibers in the frozen-defrosted whole birds had the capacity to retain the reabsorbed water, since shrinkages were similar for unfrozen and frozen birds.

The present data on free drip indicate that ground dark muscle, at most, shows only a slight susceptibility to drip. The water content of the white and dark muscle is quite similar, and it does not appear that the differences in the susceptibility of the two types of meat to drip can be attributed to differences in total moisture. In consideration of available data it seems logical to assume that the chemical and physical properties of the dark muscle favor more rapid reabsorption of the "frozen out" water. The pH values (Fig. 3) of the white meat are considerably lower than those for the dark meat. Empey (1933) found that maximum amount of drip was obtained from beef when the pH values were low and that minimum drip was obtained at a pH of 6.3 or higher. Whether the remarkable reabsorptive capacity exhibited by the dark muscle is due to the higher pH values or whether the relationship is merely incidental has not been determined for poultry.

In considering factors that are responsible for determining the susceptibility of ground poultry meat to drip, it becomes apparent that any factor that inhibits the uptake or reabsorption of the "frozen out" water tends to increase drip. The freezing temperature is a factor that may affect the amount of drip in that it will determine the size, distribution, and number of ice formations. Fast freezing favors reabsorption of the water in that it creates a maximum fiber-water surface relationship in the defrosting tissue. Regardless of the temperature of freezing, however, frozen-defrosted whole birds do not drip significantly because the area of cut surface to volume of meat is extremely small. This gives the fibers an opportunity to completely reabsorb the displaced water. If the physical and chemical properties of the defrosting muscle tissue particularly favor reabsorption, as in the case of the ground dark muscle, the rate of freezing will have little or no effect in influencing the amount of drip even though the area of cut surface is large in relation to volume of meat.

CONCLUSIONS

Similar cooking shrinkages were obtained from unfrozen birds and birds frozen at —45.5°C.(—50°F.), —26.1°C.(—15°F.), and —13.3°C.(8°F.).

As measured by free drip, ground white meat shows a significantly greater susceptibility to drip than ground dark meat.

White meat, when ground and frozen within three hours after slaughter, shows a definite susceptibility to drip, but the free drip appears to bear little or no relationship to freezing temperatures.

White meat, when ground and frozen 24 hours after slaughter, drips more when frozen at 8°F. than when frozen at —50°F.

The pH values of the dark meat are considerably higher than the values for white meat.

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PREPARATION AND USE OF LOW-LACTOSE MILK

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The main factor limiting the amount of serum solids usable in ice cream has been the lactose concentration in the serum. The low solubility of lactose in the hardened ice cream leads to a sandy or crystallized condition when an excess of milk-solids-not-fat is used. Consequently, commercial mixes seldom are made to contain more than 11 per cent of normal serum solids unless the total solids are low or it is expected that the ice cream will be consumed soon after its manufacture. An additional limit to the amount of serum solids that can be used in ice cream is the fact that increasing these solids beyond 12 per cent is likely to cause a salty flavor and a depressed freezing point.

Consequently, a practical method for removing a part of the lactose and salts normally contained in the serum portion of the mix should be of interest to the industry. Certain commercial products have been developed in which the lactose supposedly has been removed in part. Webb and Williams (1934) have presented a method for removing a portion of the lactose in sweetened condensed milk by a centrifuge or filter press.

The present study has to do with a method of preparing low-lactose milk by precipitating the colloidal casein in skim milk in such a manner that the whey and its solids can be separated. In an earlier study (1931), using Hygell as a stabilizer in ice cream, McCown noted that there occurred a shrinkage of the casein causing a whey separation. When Hygell was added to skim milk a distinct separation took place. Upon replacing the whey portion with water and mixing with the caseinous precipitate, a normal-appearing mixture resulted, indicating the casein to be in a form differing from that which results from the action of acid or rennet on milk. Thinking that the principle of this reaction might be applied to the manufacture of a special milk concentrate for use in ice cream, plans for the present study were made.

PREPARATION OF LOW-LACTOSE MILK

The material used in this study for destabilizing the protein was a commercial product called Hygell, previously referred to, which is derived through special treatment and refinement of the carob bean,

locust bean, or St. John's Bread Seeds. It is a white powder, odorless and tasteless. According to Williams (1928) the composition of the endosperm of the locust bean is as follows:

Galactin	29.18 per cent
Mannan	58.42 per cent
(Laevulan)	Trace
Pentosans	2.75 per cent
Proteins	5.29 per cent
Nitrogen	0.83 per cent
Mineral matter	0.82 per cent
Cellular tissue	3.64 per cent.

Hygell forms a pseudo solution or fine suspension in water and will produce a gel if present in sufficient quantity. Borates increase the gel action of the Hygell, whereas caustic soda and acids at high temperature will reduce the viscosity of water solutions of Hygell. According to Chittenden,[1] Hygell was used as a stabilizer in ice cream as early as 1927. The company which he represents also reports that Hygell has been used in jams and preserves, in bakery goods as a supplement to eggs, in the manufacture of processed and cream cheese, and in the making of bread.

Hygell suspensions are fine enough to pass through commercial filter paper but separation can be obtained by use of a Seitz filter. The filtrate has a viscosity comparable with water and does not thicken upon the addition of borax, indicating a separation of the gum has taken place. The destabilizing action of Hygell upon casein is not fully understood but is likely one of dehydration.

In an early study of the use of Hygell as a stabilizer in ice cream, where it was desired to prevent the whey separation, it was found that when varying percentages of sodium bicarbonate, sodium phosphate, sodium phosphite, sodium citrate, calcium lactate, calcium oxide, and aluminum chloride were used, no deterrent effect was noticeable. In all cases the addition of these salts decreased the viscosity slightly, and at times the rapidity of the separation was accelerated. It has been found that a calcium salt sometimes aids in obtaining a clearer whey and a more distinct separation and for that reason its use is included in the following procedure for making the low-lactose milk.

1. Pasteurize the skim milk by heating to 71.1°C.(160°F.) for 20 minutes.
2. While the milk temperature is being raised, add three ounces of Hygell for each 100 pounds of skim milk. The Hygell is sprinkled into the skim milk while it is being vigorously agitated.
3. Cool the pasteurized skim milk to 32.2°C.(90°F.) or lower and add one pint of a 10 per cent solution of calcium chloride for each 100 pounds of skim milk. Continue cooling to below 4.4°C.(40°F.).

[1] Personal communication.

4. Leave mixture in a quiescent state in a closed insulated vat until a separation of the casein and whey takes place. This will require from 12 to 48 hours.

5. The lower layer, which contains largely the precipitated casein, is separated from the whey portion by permitting it to flow through the discharge valve. This will include approximately one-fifth of the original volume of skim milk.

It is the lower portion that contains the reduced percentage of lactose. The total-solids content has been found to vary from 15 to 17 per cent. An analysis of one batch gave the following:

Protein	10.1 per cent
Lactose	4.2 per cent
Other solids	1.5 per cent
Total solids	15.8 per cent

Skim milk with a total-solids content of nine per cent contains approximately 3.3 per cent protein and 4.95 per cent lactose. The relative proportion of protein to lactose in the low-lactose milk is 2.405 to 1, whereas in the case of skim milk the proportion is .66 to 1. In other words, there is approximately one-fourth as much lactose per given weight of protein in the low-lactose milk as there is in the case of the normal skim milk. Thus it becomes possible to use a high percentage of serum solids in the mix when using low-lactose milk without getting too high a lactose content in the ice cream.

USE OF LOW-LACTOSE MILK IN ICE CREAM

Ordinarily it would not be possible to secure a sufficient amount of serum solids to produce the desired improvement of body by using low-lactose milk and cream as the only milk products in the mix. It is advisable, therefore, to use some type of normal condensed milk along with the low-lactose milk. A number of experimental mixes were prepared, using various combinations of normal and low-lactose serum solids. It was found that if more than four per cent of low-lactose solids was used in a mix containing as much as 13 per cent serum solids, the ice cream body was criticized for being slightly dry. For a 12-per cent fat ice cream, a combination of nine per cent serum solids and four per cent low-lactose solids was found most desirable. An ice cream containing 12 per cent fat, nine per cent normal serum solids, four per cent low-lactose solids, and 15 per cent sugar had a smoother and more resistant body and equally as good a flavor as the control ice cream containing 11 per cent of normal serum solids.

Since part of the Hygell is carried into the low-lactose milk, it is advisable to reduce the gelatin content about 30 per cent when using the product.

If it is desired to increase the percentage of low-lactose solids beyond four, the dry type of body that results can be overcome to a certain extent by replacing 25 per cent of the cane sugar with anhydrous dextrose.

In a number of trials in which comparisons were made between the control ice cream containing 12 per cent fat, 11 per cent serum solids, 15 per cent sugar, and .35 per cent gelatin and the experimental ice cream containing 12 per cent fat, 9 per cent normal serum solids, 4 per cent low-lactose solids, 15 per cent sugar, and .25 per cent gelatin it was found that the latter had a better body and superior flavor. The ice cream containing the low-lactose solids has the appearance, flavor, and body of an ice cream higher in butterfat than it actually is.

Mixes containing low-lactose solids whip slightly slower than those containing all normal serum solids. In melting tests, conducted at room temperature, ice creams containing low-lactose solids stood up slightly better than the control samples.

USE OF LOW-LACTOSE MILK AND DEXTROSE SUGARS

One of the objections to the use of monosaccharide sugars, such as dextrose, levulose, or honey, in ice cream has been the lowering effect upon the freezing point of the ice cream necessitating a somewhat lower hardening room and dispensing temperature. Since lactose is one of the crystalloids responsible for lowering the freezing point in ice cream, it is reasonable to expect that removing part of the lactose normally present in ice cream would offset some of the depressing effect upon the freezing point when a dextrose sugar replaces sucrose. To study this possibility the following mixes were prepared:

Mix A—12 per cent fat
11 per cent serum solids
15 per cent sugar
0.35 per cent gelatin (225 bloom)
0.30 per cent egg yolks.

Mix B—12 per cent fat
5.5 per cent normal serum solids
5.5 per cent low-lactose serum solids
11.25 per cent cane sugar
3.75 per cent anhydrous dextrose
0.30 per cent gelatin
0.30 per cent egg yolks.

The viscosity of the mix containing the normal serum solids was 82 at 4.4°C.(40°F.), using the MacMichael Viscosimeter with a No.

26 wire, while the viscosity of the mix containing the low-lactose solids was 155. The scores of the ice creams were as follows:

	Flavor	Body
Normal solids ice cream	43.75	23.5
Low-lactose solids ice cream	45.00	24.0.

The normal-solids ice cream was criticized as having a noticeable serum-solids flavor, whereas the ice cream with low-lactose solids was considered as being richer in flavor and cleaned up very well in the mouth. The body of the low-lactose solids ice cream was superior because of its greater smoothness and similarity to the body of a high-fat product.

TABLE 1

Effect of Low-Lactose Solids Upon Freezing Process Using Batch Freezer

MIX A[1]		Minutes	Mix B[1]	
Temperature	Overrun		Temperature	Overrun
°F.	pct.		°F.	pct.
40.8	.....	0	39.8	.....
28.1	20	1	27.5	17
26.1	27	2	25.5	26
24.9	43	3	25.0	40
24.1	48	4	24.1	49
23.6	63	5	23.7	62
23.8	82	6	24.0	77
24.0	98	7	24.1	86
.....	104	8	24.4	97
.....	115	9	.....	103
.....	116	10	.....	108

[1] Refrigeration off after three minutes and 20 seconds.

Observations made on a number of ice creams containing 12 per cent fat, 15 per cent sugar, and 13 per cent serum solids showed that when three or more of the 13 per cent serum solids were low-lactose solids, sandiness did not develop even after two months' storage in the hardening room.

UTILIZATION OF WHEY PORTION

The cost of low-lactose milk depends largely upon the value placed on the skim milk and the possibilities of utilizing the whey. Assuming the skim milk to be worth 35 cents per hundred and the gum to be available at 50 cents per pound, the material cost for a pound of the low-lactose solids would be approximately 14 cents. From this cost would need to be deducted the saving in stabilizer and possible value of the whey portion.

Where facilities permit, the whey portion may be utilized in the manufacture of starter distillate, as recommended by Ruehe and

Ramsey (1935). Trial runs have shown that larger yields of diacetyl can be obtained from whey starter than from regular skim-milk starter.

The ripened whey, when standardized to two per cent butterfat by the addition of sweet cream, makes an extremely palatable, low-protein, light-bodied, cultured milk drink. It seems likely that a drink of this type would meet the approval of a good many consumers, particularly those desiring a low-protein diet.

The whey portion has also been found to have unusually good whipping properties and when whipped has somewhat the same appearance and consistency as whipped egg albumen. A means for utilizing the whey in this manner was not determined.

SUMMARY

A method is presented for making a low-lactose milk to be used in ice cream. By the use of this product ice cream containing nine per cent normal serum solids and four per cent low-lactose solids can be made that will not become sandy. Such a product has a superior body and flavor to ice cream containing the usual amount of normal serum solids. Replacing approximately one-half of the normal serum solids in a mix with the low-lactose solids, when 25 per cent of the sugar in a mix has been replaced with anhydrous dextrose, results in an ice cream with a somewhat higher drawing temperature. The ice cream in this case has a superior flavor and body. Possible commercial uses of the whey fraction are mentioned.

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EXPERIMENTAL WORK ON LACTIC ACID IN PRESERVING PICKLES AND PICKLE PRODUCTS

I. RATE OF PENETRATION OF ACETIC AND LACTIC ACIDS IN PICKLES[1]

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It has been observed by Fabian, Bryan, and Etchells (1932) that during the normal fermentation of cucumber salt stock the ratio of non-volatile acid as lactic to volatile acid as acetic was approximately 6 : 1 in 30° salometer brine and 5 : 1 in 40° salometer brine.

In the work of Fabian and Wickerham (1935) with genuine dill pickles it was found that the ratio of lactic to acetic acid was even higher, 8 : 1. Lactic acid is likewise the predominant acid in many dairy products, such as buttermilk, butter, and cheese. In the case of sauerkraut, again lactic is the principal acid.

A review of the literature on the physiological effects of lactic and acetic acids by Fabian (1939) shows that the body mechanism is capable of utilizing these acids in quantities normally found in foods without any pathological effects on the organs or without any undue stress being placed upon the alkaline reserve of the blood or the organs of elimination. In fact, both acids are used in infant feeding to reduce the buffering effect of cow's milk.

Furthermore, it has been shown recently by Cori and Cori (1929) that lactic acid is utilized by the body in the formation of liver glycogen, and by Lusk (1921) that acetic acid is oxidized in the body, affording a readily available source of energy. Since lactic and acetic acids are produced in varying ratios during the normal process of fermentation in many food products, a study has been made of various combinations of these two acids to test their suitability for use in connection with pickles and pickle products.

In a series of experiments such as these, three things must be taken into consideration. The first, and paramount, is the influence of the preservatives on keeping quality of the product; second, the effect of the preservative on flavor of the product; and finally, the cost.

FRESHENING PROCESS

The freshening process is the first step in the manufacture of pickles or pickle products from cucumber salt stock. For the ex-

[1] Journal article No. 350 N. S.; this paper is the first in a series called "Experimental Work on Lactic Acid in Preserving Pickles and Pickle Products."

TABLE 1

Rate at Which NaCl Is Withdrawn From Small-Sized Pickles Taken From 46-Degree Salometer Brine[1] During Freshening Process

Date	Treatment	Salometer	Liquor			Pickle		
			NaCl	Acetic acid	pH	NaCl	Acetic acid	pH
			pct.	*pct.*		*pct.*	*pct.*	
Batch No. 1								
4/36	Original brine	46	12.39	.30	4.00	12.60	.35	4.00
4/36	1 hr. after first heating in water	24	6.60	.20	4.20	6.20	.36	4.32
4/36	1 hr. after second heating in water	20	5.75	.20	4.23	5.60	.35	4.57
4/36	24 hr. after second heating in water	21	5.92	.10	4.15	6.10	.28	4.40
4/36	24 hr. after soaking in turmeric and alum and changing water	12	3.40	.20	3.60	2.97	.24	4.00
Batch No. 2								
4/36	Original brine	46	12.39	.30	4.00	12.60	.35	4.00
4/36	1 hr. after first heating in water	24	6.60	.20	4.20	6.55	.33	4.10
4/36	1 hr. after second heating in water	20.5	5.50	.20	4.30	5.00	.35	4.49
4/36	24 hr. after second heating in water	21	5.70	.10	4.06	6.00	.28	4.30
4/36	24 hr. after soaking in turmeric and alum and changing water	11	2.90	.25	3.60	2.97	.24	4.00

[1] 12.39 per cent salt.

perimental work, pickles were removed from the brine in quantities of approximately one bushel and placed in 30-gallon crocks. Water was added to the pickles at the rate of approximately 67 pounds (eight gallons) of water to 50 pounds (one bushel) of salt stock. Steam was introduced at such a rate that the temperature rose to 43.3°C.(110°F.) in one hour. The freshening stock was allowed to cool to room temperature after which it was again heated slowly to 48.8°C.(120°F.), allowed to cool and remain in the crock for 24 hours, and drained off. The same quantity of water was added to the stock a second time and in addition alum $Al_2(SO_4)_3 \cdot 18H_2O$ at the rate of one pound per three bushels and turmeric at the rate of one pound per 200 gallons of water and pickles. At the end of 24 hours this water was drained off and the stock placed in the same quantity of fresh water for one hour to remove the excess alum and turmeric. The pickles were then ready for the acid treatment.

The rate at which sodium chloride diffuses out of small-sized pickles (National Pickle Packers No. 1 grade) is shown (Table 1); the rate of diffusion from medium-sized pickles (National Pickle Packers No. 2 grade) is given (Table 2). The results show that with this method of treatment, about 50 per cent of the salt is withdrawn from both sizes of pickles within the first two hours. The rate at which salt was withdrawn during the next 22 hours was much slower since the pickles and the liquor in which they were soaking had reached nearly equilibrium. After reheating, there was a slight increase in the amount of salt removed but at a much slower rate than after the first heating. At the end of the second 24 hours the amount of salt removed increased but at a slower rate than during the first 24-hour freshening period, because the concentration of the salt was less and equilibrium was gradually being attained.

The salt was withdrawn at a faster rate from the small than from the medium-sized pickles. These results indicate that the freshening period may be greatly shortened, thereby saving time and improving both the quality and firmness of the pickles as well as reducing the hazards of spoilage. Likewise, the second heating was of small value and could be dispensed with. More recent work by Switzer, Richardson, and Fabian (1939) has shown that 95 per cent of the salt is withdrawn from the pickles within the first eight hours at temperatures of 21.1°C.(70°F.) or above. Salt pickles can be freshened in eight to ten hours by circulating fresh water through the tanks at a moderate rate.

ACIDIFYING PROCESS

After the pickles were freshened, they were acidified. For this purpose acetic and lactic acids (Table 3) and a combination of them

TABLE 2

Rate at Which NaCl Is Withdrawn From Small-Sized Pickles Taken From 42-Degree Salometer Brine[1] During Freshening Process

Date	Treatment	Salometer	Liquor			Pickle		
			NaCl	Acetic acid	pH	NaCl	Acetic acid	pH
			pct.	*pct.*		*pct.*	*pct.*	
Batch No. 1								
4/1/36	Original brine	42	11.50	.40	3.55	10.15	.52	3.70
4/2/36	1 hr. after first heating in water	21	5.60	.25	3.81	7.00	.52	4.40
4/2/36	1 hr. after second heating in water	16	5.37	.27	3.90	5.70	.52	4.40
4/3/36	24 hr. after second heating in water	10	6.10	.10	4.00	5.07	.24	4.00
4/4/36	24 hr. after soaking in turmeric and alum and changing water	8	2.10	.18	3.64	3.50	.28	4.00
Batch No. 2								
4/2/36	Original brine	42	11.50	.40	3.55	10.15	.52	3.70
4/2/36	1 hr. after first heating in water	10	2.60	.28	4.57	7.00	.45	4.06
4/3/36	1 hr. after second heating in water	10	3.45	.07	4.06	5.27	.28	3.80
4/4/36	24 hr. after second heating in water	15	4.30	.10	3.90	4.25	.17	4.00
4/5/36	24 hr. after soaking in turmeric and alum and changing water	6	1.40	.20	3.90	3.15	.35	3.38
Batch No. 3								
4/2/36	Original brine	42	11.50	.40	3.55	10.15	.52	3.70
4/2/36	1 hr. after first heating in water	10	2.50	.35	4.49	7.50	.35	4.00
4/3/36	1 hr. after second heating in water	12	3.70	.10	3.90	6.30	.24	3.90
4/4/36	24 hr. after second heating in water	14	4.00	.10	3.90	4.02	.17	4.06
4/5/36	24 hr. after soaking in turmeric and alum and changing water	5	1.15	.22	3.80	2.80	.35	3.38

[1] 11.50 per cent salt.

TABLE 3

Rate of Penetration of Different Percentages of Acetic and Lactic Acids Into Medium-Sized Pickles

Time	Acetic acid in liquor	Difference [1]	Absorbed [2]	pH
	Batch No. 1—5 per cent acetic acid			
days	*pct.*		*pct.*	
0	5.10	0	0	.....
1	3.87	1.23	67.5	2.70
2	3.50	1.60	88.0	2.78
3	3.42	1.68	92.3	2.84
5	3.34	1.76	96.6	2.88
7	3.28	1.82	100.0	2.90
	Batch No. 2—4.5 per cent acetic acid			
0	4.42	0	0	.....
1	4.10	0.32	39.0	2.67
2	3.96	0.46	56.0	2.71
3	3.91	0.51	62.2	2.61
5	3.80	0.62	75.7	2.80
7	3.60	0.82	100.0	2.75
	Batch No. 3—4 per cent acetic acid			
0	4.07	0	0	.....
1	3.65	0.42	40.4	2.70
2	3.57	0.50	48.0	2.70
3	3.39	0.68	65.4	2.63
5	3.12	0.95	91.3	2.82
7	3.03	1.04	100.0	2.80

Time	Lactic acid as acetic	Difference [1]	Absorbed [2]	pH	Remarks
	Batch No. 4—5 per cent lactic acid				
days	*pct.*		*pct.*		
0	3.32	0	0	.....	.....
1	3.07	.25	29.4	2.12	.....
2	2.78	.54	63.6	2.18	.....
3	2.73	.59	69.4	2.05	.....
5	2.50	.82	96.5	2.20	[3]
7	2.47	.85	100.0	2.20	[4]
	Batch No. 5—4.5 per cent lactic acid				
0	3.00	0	0	.....	.....
1	2.87	.13	17.3	2.12	[5]
2	2.60	.40	53.4	2.14	[5]
3	2.46	.54	72.0	2.09	[5]
5	2.38	.62	82.7	2.20	[6]
7	2.25	.75	100.0	2.18	[6]
	Batch No. 6—4 per cent lactic acid				
0	2.65	0	0	.....	.....
1	2.50	.15	25.0	2.21	[5]
2	2.42	.23	38.3	2.14	[5]
3	2.29	.36	60.0	2.12	[3]
5	2.10	.55	91.6	2.20	[3]
7	2.05	.60	100.0	2.20	[6]

[1] Difference between initial concentration of acid and concentration of acid left in solution. [2] Per cent of total amount of acid capable of being absorbed by the pickle, i.e., 100 per cent represents equilibrium between acid in the pickle and acid in the liquor surrounding the pickle. [3] Mycoderma scum, pickles slightly slippery. [4] Pickles slippery and soft throughout. [5] Pickles soft. [6] Mycoderma scum, pickles slippery and slightly soft.

in varying proportions (Table 4) were used in concentrations of 4, 4.5, and 5 per cent.

A study was made of the rate of penetration of the two acids and various combinations of them during the course of the acidifying process; the data on some of these experiments showing representative results are given (Tables 3 and 4). The method used was to add equal amounts of liquor and pickles, usually 100 pounds of each, to large crocks and then to titrate the liquor at stated time intervals and remove a representative pickle at the same time. The

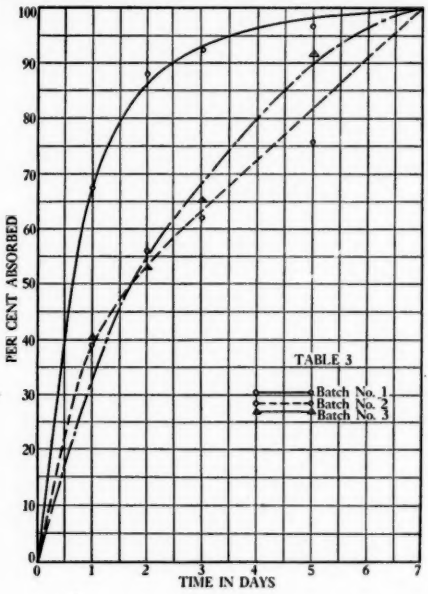

FIG. 1

pickles were sliced, 10 grams of the slices from different sections were ground in a mortar and pestle with 40 ml. of distilled water, and this infusion was allowed to remain for an hour. Five milliliters were then titrated and the amount of acid which had diffused into the pickle was determined. It was found with this technic that not only pickles of different sizes, but also pickles of the same size as well as different portions of the pickle, absorbed different amounts of acid. The results obtained by an analysis of the pickles by this method are not included since they give no additional information not shown by an analysis of the liquor.

It will be observed that acetic acid (Fig. 1) penetrates the pickles at a faster rate than does lactic acid (Fig. 2). The pickles had ab-

TABLE 4

Rate of Penetration of Different Combinations of Acetic and Lactic Acids Into Medium-Sized Pickles

Time	Acetic acid in liquor	Difference [1]	Absorbed [2]	pH
	Batch No. 7—4.5 per cent acetic and .5 per cent lactic			
days	*pct.*		*pct.*	
0	4.85	0	0	2.58
1	4.00	0.85	52.5	2.46
2	3.85	1.00	61.7	2.54
3	3.73	1.12	69.1	2.46
5	3.60	1.25	77.1	2.65
7	3.39	1.46	90.0	2.63
13	3.23	1.62	100.0	2.71
	Batch No. 8—4 per cent acetic and 1 per cent lactic			
0	4.65	0	0	2.46
1	3.37	1.28	59.0	2.46
2	2.82	1.83	84.3	2.54
3	2.80	1.85	85.3	2.56
5	2.60	2.05	94.5	2.71
7	2.58	2.07	95.4	2.69
13	2.48	2.17	100.0	2.71
	Batch No. 9—3.5 per cent acetic and 1.5 per cent lactic			
0	4.41	0	0	2.37
1	3.18	1.23	63.6	2.40
2	2.68	1.73	89.6	2.53
3	2.67	1.74	90.0	2.50
5	2.62	1.79	92.7	2.52
7	2.50	1.91	99.0	2.62
13	2.48	1.93	100.0	2.63

Time	Lactic acid as acetic	Difference [1]	Absorbed [2]	pH
	Batch No. 10—4 per cent acetic and .5 per cent lactic acid			
days	*pct.*		*pct.*	
0	4.27	0	0	2.46
1	2.70	1.57	79.6	2.63
2	2.60	1.67	84.8	2.71
3	2.50	1.77	89.9	2.56
5	2.46	1.81	91.9	2.66
7	2.33	1.94	98.4	2.80
13	2.30	1.97	100.0	2.80
	Batch No. 11—3.5 per cent acetic and 1 per cent lactic			
0	4.10	0	0	2.40
1	2.55	1.55	71.0	2.54
2	2.40	1.70	77.9	2.60
3	2.18	1.92	88.0	2.60
5	2.12	1.98	90.8	2.67
7	2.10	2.00	91.7	2.77
13	1.92	2.18	100.0	2.77
	Batch No. 12—3 per cent acetic and 1.5 per cent lactic acid			
0	3.93	0	0	2.37
1	2.70	1.23	62.7	2.46
2	2.27	1.66	84.6	2.63
3	2.15	1.78	90.8	2.56
5	2.12	1.81	92.4	2.69
7	2.08	1.85	94.4	2.69
13	1.97	1.96	100.0	2.71

[1] Difference between initial concentration of acid and concentration of acid left in solution. [2] Per cent of total amount of acid capable of being absorbed by the pickle, i.e., 100 per cent represents equilibrium between acid in the pickle and acid in the liquor surrounding the pickle.

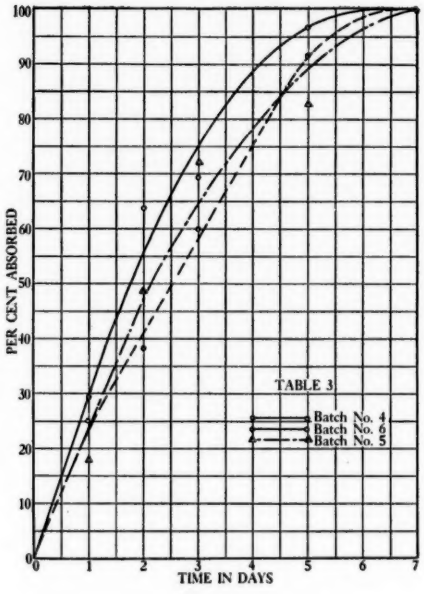

FIG. 2

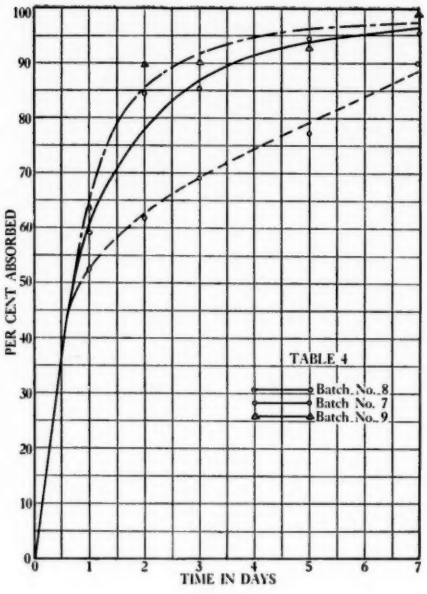

FIG. 3

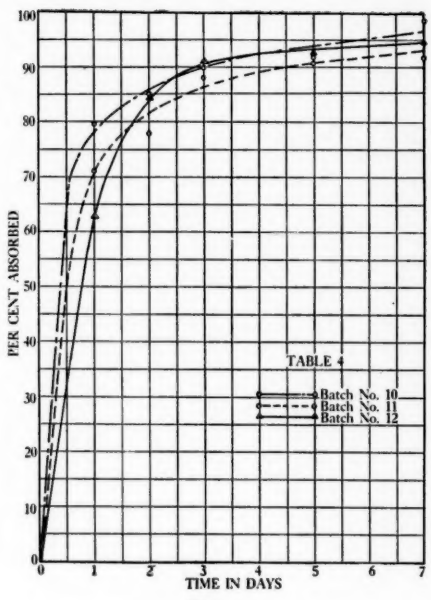

FIG. 4

sorbed 40 per cent or more of the total amount of acetic acid which they were capable of absorbing within the first 24 hours after they were placed in the acid liquor. After the first 24 hours the acid penetrated more slowly. It required about seven days for an equilibrium to be established between the pickles and the acid liquor in which they were placed. The rate of penetration depended to a less degree also upon concentration of the acid and was not as fast with four per cent acetic acid as with five per cent.

The rate of penetration of lactic acid into the pickles is slower than for acetic acid since two days elapsed before 40 per cent or more of the acid had been absorbed by the pickle.

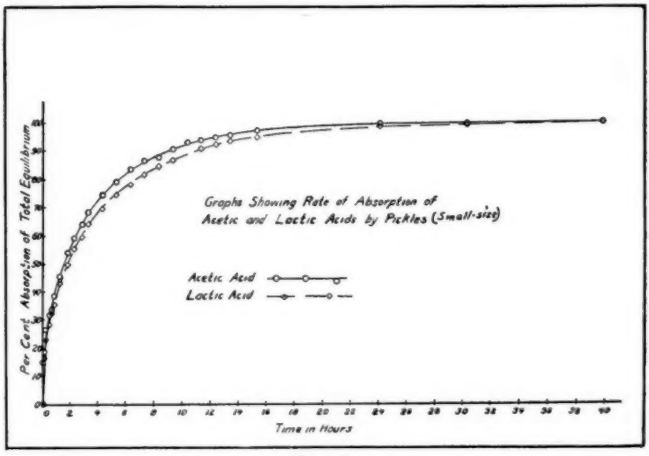
Per Cent Absorption of Total Equilibrium
Graphs Showing Rate of Absorption of Acetic and Lactic Acids by Pickles (Small-size)
Acetic Acid
Lactic Acid
Time in Hours

FIG. 5

Combining acetic and lactic acids increased the rate of penetration of the acid into the pickles (Table 4 and Figs. 3 and 4), since there was a 50-per cent absorption within the first 24 hours. In fact, in all cases except one (five per cent acetic acid, Batch No. 1, Table 3) there was a more rapid penetration in the first 24-hour period when the two acids were combined than when the acids were used alone.

The slow rate of penetration of lactic acid (Table 3) may or may not explain why the pickles became slippery and in some instances turned soft. The growth of Mycoderma on some of the jars certainly indicates the lack of preserving power of the lactic acid in these concentrations.

Additional experiments were run with acetic and lactic acids, using small pickles and a shorter time interval, in order to deter-

mine just how soon equilibrium is reached between the pickle and the acid liquor (Fig. 5). The graph shows clearly that acetic acid penetrates the pickle more readily than does lactic acid. It also shows that with small pickles the rate of absorption is greatest during the first six hours after the pickles are placed in the acid liquor, since from 75 to 80 per cent of the total amount of acid that is absorbed is absorbed in this time, whereas the rest of the acid is absorbed in the remaining 34 hours. It also shows that it is not necessary to keep small pickles in the acid liquor longer than 40 hours.

In this connection it should be noted that not only the concentration of acid but also the ratio of pickles to acid is important. When equal quantities by weight of pickles and liquor were used, it was found that with acetic acid approximate equilibrium was reached within 24 hours. Absorption is most rapid during the first hour and one-half, at which time the pickles have taken up 50 per cent of the total amount of acid which they will absorb. From that time on the rate of absorption is slower as the point of equilibrium is approached.

When lactic acid was used as the first acidifying medium after the pickles had been freshened, after five days the pickles developed "slips" and at the end of seven days they were soft (Table 3). When 4.5 per cent lactic acid was used, the pickles were soft at the end of 24 hours, as was also the case when four per cent lactic acid was used. For this reason it was evident that lactic acid could not be used for the first acidifying medium for freshened cucumber stock. A combination of acetic and lactic acids was used with satisfactory results (Table 4).

GENERAL DISCUSSION

A comparison of the rate at which salt is withdrawn with the rate at which it penetrates the pickle is significant. According to Campbell (1920) cucumbers absorb from six to eight per cent salt during the first 36 hours after they are placed in the brine. The results (Tables 1 and 2) show that approximately 50 per cent of the total amount of salt in the pickle was withdrawn within two hours, and that it was withdrawn at a faster rate from small-sized than from medium-sized pickles. Heat was used but the salt is withdrawn nearly as rapidly without heat as with heat, we have since determined. This indicates that the physical condition of the fresh cucumber has been greatly altered during the salting process.

SUMMARY

The results show that salt is removed at a much faster rate during the first than during the second 24-hour freshening period. Approximately 50 per cent of the salt was removed during the first hour after heating. A second heating was of little value in facilitating the removal of salt. Salt is removed from small pickles more rapidly than from medium-sized ones.

Acetic acid penetrates pickles more rapidly than lactic acid.

The rate of penetration of acid into the pickles is greatest during the first 24 hours after they are placed in the acid liquor. In the case of small pickles 75 to 80 per cent of the total amount of acid absorbed by them is absorbed in the first six hours after they are placed in the acid liquor. Equilibrium is reached within 40 hours for small pickles, whereas with medium-sized pickles it requires a longer time. Not only is the concentration of the acid important but also the ratio of weight of the pickles to weight of the acid present.

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EXPERIMENTAL WORK ON LACTIC ACID IN PRESERVING PICKLES AND PICKLE PRODUCTS

II. PRESERVING VALUE OF ACETIC AND LACTIC ACIDS IN THE PRESENCE OF SUCROSE[1]

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Previous studies by Fabian and Wadsworth (1939) showed that acetic acid penetrated processed pickles more rapidly than lactic acid. Additional experiments were run to determine the preserving action of acetic and lactic acids separately and also combinations of the two acids in the presence of sucrose. In the preliminary experiments, as well as in the final experiments, an aciduric yeast was used, since experience of the authors with acid foods over a period of years has demonstrated that acid-tolerant yeasts cause most of the trouble. The yeast selected was one isolated by Ayers (1926) from catsup. This yeast is very resistant to acid, salt, and sugar. Pederson and Breed (1926) found that it would grow in catsup containing 1.32 per cent acetic acid, 19.2 per cent sugar, and 3.8 per cent salt. Before inoculation the acid tolerance of the yeast was tested and found to be as reported.

For the preliminary experiments pickles that had been previously prepared by acidification with acetic acid were placed in the combinations of acid and sucrose (Tables 1, 2, and 3) and allowed to remain in this liquor until equilibrium between the pickle and liquor was established. They were then canned in duplicate quart Mason jars and inoculated with a broth culture of an acid-resisting yeast.

For the final experiments the pickles were treated the same as in the preliminary experiments except that they were placed in jars and allowed to stand for about a week, after which they were tested for the presence of microörganisms by plating one-ml. quantities of the liquor on maltose extract agar before inoculation. The jars were then inoculated with the above yeast, and tests for the presence of the yeast and other indications of spoilage were made by plating on the above medium over a period of 20 weeks (Tables 1, 2, and 3). In the last two columns of the tables are shown the number of sam-

[1] Journal article No. 350 N. S.

TABLE 1

Preserving Value of Acetic Acid in Combination With Sucrose for Pickles Inoculated With an Aciduric Yeast

1	2	3	6	7	8	9	10	11	12	13	14	15	16	17		
No.	Date canned	Acetic acid (per cent)			pH 10/11/36	Baumé reading (degrees)		Test for presence of yeast							No. +	No. —
		Calculated	Titratable acidity			Calculated	Actual when canned	Before inoculation	Time after inoculation							
			When canned	10/11/36					1 week	2 weeks	4 weeks	7 weeks	14 weeks	20 weeks		
36	6/20/36	1.60	1.58	1.53	2.63	14.0	14.2	— +	— +	— +	— —	+ —	+ +	— —	5	7
37	6/20/36	1.60	1.57	1.51	2.71	16.0	16.0	— —	— —	— +	— —	—	—	— —	1	9
38	6/20/36	1.60	1.59	1.53	2.75	18.0	18.0	— —	— +	— —	— +	— +	— —	— —	3	9
39	6/20/36	1.60	1.55	1.51	2.73	20.0	20.0	— —	— —	— —	— —	— —	— —	— —	0	12
40	6/20/36	1.60	1.57	1.49	2.71	22.0	22.0	— —	+ —	— —	— —	— —	— —	— —	1	11
41	6/20/36	1.60	1.57	1.51	2.71	24.0	24.0	— —	— —	— +	— —	— —	— —	— —	1	11
90	6/1/36	1.80	1.80	1.70	2.97	14.0	13.5	— —	— +	.....	.....	+ —	+	— +	3	4
91	6/1/36	1.80	1.80	1.70	2.92	16.0	16.2	— —	— —	.....	.....	— —	— —	— —	0	8
92	6/1/36	1.80	1.82	1.80	2.88	18.0	18.0	— —	— —	.....	.....	— —	— +	+ —	2	6
93	6/1/36	1.80	1.81	1.78	2.88	20.0	20.5	— —	— —	.....	.....	— —	— —	— —	0	8
94	6/1/36	1.80	1.79	1.71	2.80	22.0	22.0	— —	— —	.....	.....	— —	— —	— —	0	8
95	6/1/36	1.80	1.80	1.69	2.80	24.0	23.7	— —	— —	.....	.....	— —	— —	— —	0	8
60	5/17/36	2.00	2.03	1.95	2.63	14.0	14.0	— —	— +	— +	— —	— —	— —	— —	2	10
61	5/17/36	2.00	1.95	1.88	2.63	16.0	16.0	— —	— +	— +	— —	— —	— —	— —	2	10
62	5/17/36	2.00	2.00	1.87	2.58	18.0	18.0	— —	— +	— —	— —	— —	— —	— —	1	11
63	5/17/36	2.00	2.00	1.92	2.54	20.0	20.2	— —	— —	— —	— —	— —	— —	— —	0	12
64	5/17/36	2.00	2.00	1.98	2.54	22.0	22.0	— —	— —	— —	— —	— —	— —	— —	0	12
65	5/17/36	2.00	1.98	1.83	2.50	24.0	24.5	— —	— —	— —	— —	— —	— —	— —	0	12
66	4/28/36	2.20	2.21	2.18	2.54	14.0	14.5	— +	— +	— —	— —	— —	— —	— —	1	11
67	4/28/36	2.20	2.21	2.19	2.54	16.0	16.0	— —	+ +	— —	— —	— —	— —	— —	2	10
68	4/28/36	2.20	2.18	2.10	2.54	18.0	18.0	— —	+ —	+ —	— —	— —	— —	— —	2	10
69	4/28/36	2.20	2.22	2.14	2.48	20.0	20.2	— —	— —	— —	— —	— —	— —	— —	0	12
70	4/28/36	2.20	2.23	2.16	2.48	22.0	21.8	— —	— —	— —	— —	— —	— —	— —	0	12
71	4/28/36	2.20	2.19	2.00	2.46	24.0	23.9	— —	— —	— —	— —	— —	— —	— —	0	12
78	4/28/36	2.40	2.30	2.26	2.54	14.0	13.9	— —	— +	— —	— —	— —	+ —	+ —	3	9
80	4/28/36	2.40	2.40	2.30	2.50	18.0	17.9	— —	— —	— —	— —	— —	— —	— —	0	12
81	4/28/36	2.40	2.39	2.30	2.50	20.0	20.0	— —	— —	— —	— —	— —	— —	— —	0	12
82	4/28/36	2.40	2.34	2.22	2.46	22.0	22.1	— —	— +	— —	— —	— —	— —	— —	1	11
83	4/28/36	2.40	2.39	2.20	2.46	24.0	24.1	— —	— —	— —	— —	— —	— —	— —	0	12
														Total.....	30	291
														Per cent.....	9.3	90.7

ples still showing the presence or absence of viable yeasts as determined by plating.

These tables present an excellent opportunity to observe the influence of two factors on the viability of yeasts: first, the effect of an increased sugar concentration in the presence of a constant amount of acid; and second, the effect of increasing the acid concentration in the presence of a constant amount of sugar. Information is likewise available for two acids, lactic and acetic, and combinations of the two acids in various concentrations of sucrose.

Starting with 1.6 per cent acetic acid and increasing the acidity by increments of .2 per cent, the percentage of jars showing viable yeasts irrespective of the sucrose concentration is 15.7, 10.6, 6.95, 6.95, and 6.67, respectively (Table 1). It is interesting to note that there is no decrease in the viability of yeasts after two per cent acid is reached. Analyzing the data from the standpoint of sucrose, it is found that starting with a 14-Baumé syrup, irrespective of the acid concentration, and increasing the Baumé by increments of two that there is a reduction in the number of jars showing viable yeasts from approximately 25 per cent in the case of a 14-Baumé syrup to two per cent for a 24-Baumé syrup. There was no significant reduction, however, until 22 Baumé had been reached.

Lactic acid presents a different picture (Table 2); starting at 1.4 per cent lactic acid and increasing the concentration by increments of .2 per cent up to 2.2 per cent, there is a reduction only in the number of jars showing viable yeasts from 82 per cent in the lowest acidity to 69 per cent in the highest, whereas with acetic acid the decrease was from 15.7 to 6.67 per cent, respectively. The pH in the case of lactic acid was likewise much lower than with acetic but this had no effect upon the viability of yeasts. In other words pH is not the important factor under these conditions.

The effect of increasing the amount of sucrose in the presence of lactic acid (Table 2) is to decrease the viability of the yeasts. The decrease is from approximately 70 per cent at 14 Baumé to 50 per cent at 24 Baumé as compared with from 25 to two per cent, respectively, for acetic.

When the two acids were combined in various ratios (Table 3), the viability of the yeasts was directly proportional to the amount of acetic acid present. The lactic acid had little or no effect and there was no correlation between the pH and viability of the yeasts. The percentage of jars showing viable yeasts ranged from 61.1 per cent with a mixture of 1.2 per cent acetic and .8 per cent lactic to 28.4 per cent with a mixture of 1.8 per cent acetic and .2 per cent lactic.

TABLE 2

Preserving Value of Lactic Acid in Combination With Sucrose for Pickles Inoculated With an Aciduric Yeast

No.	Date canned	Lactic acid (per cent) expressed as acetic acid			pH 10/11/36	Baumé reading (degrees)		Test for presence of yeast												No. +	No. —
		Calculated	Titratable acidity			Calculated	Actual when canned	Before inoculation		Time after inoculation											
			When canned	10/11/36						1 week		2 weeks		4 weeks		7 weeks		14 weeks		20 weeks	
1	5/12/36	1.40	1.42	1.48	2.16	14.0	14.0	—	—	+	+	+	+	+	+	+	+	+	—	11	1
2	5/12/36	1.40	1.45	1.49	2.12	16.0	16.0	+	—	+	—	+	+	+	+	+	+	+	—	10	2
3	5/12/36	1.40	1.46	1.54	2.10	18.0	18.2	+	+	—	+	+	+	+	+	+	+	+	—	10	2
4	5/12/36	1.40	1.49	1.46	2.07	20.0	20.2	+	+	+	—	+	+	—	—	+	+	—	+	7	5
5	5/12/36	1.40	1.47	1.50	2.03	22.0	22.0	+	—	+	—	+	+	+	+	+	+	—	+	10	2
6	5/12/36	1.40	1.45	1.43	2.03	24.0	23.9	+	—	+	+	+	+	+	+	.....		.....		.....		6	0
30	6/20/36	1.60	1.60	1.62	2.09	14.0	14.0	+	—	+	+	+	+	+	+	+	+	.....		+	+	10	0
31	6/20/36	1.60	1.63	1.61	2.03	16.0	16.0	+	—	+	+	+	+	+	—	+	—	+	—	+	—	8	4
32	6/20/36	1.60	1.65	1.63	1.99	18.0	18.0	—	—	—	—	—	+	—	—	—	+	+	+	+	+	6	6
33	6/20/36	1.60	1.65	1.60	1.95	20.0	20.0	—	—	+	—	+	+	+	+	+	+	+	+	+	+	11	1
12	6/4/36	1.80	1.80	1.88	1.95	14.0	13.8	—	—	—	+	—	—	—	—	—	—	—	—	—	—	1	11
13	6/4/36	1.80	1.80	1.82	1.95	16.0	16.0	—	—	+	+	+	+	—	—	+	+	+	—	—	—	7	5
14	6/4/36	1.80	1.80	1.85	1.95	18.0	18.0	—	—	—	+	—	+	—	+	—	—	+	+	+	—	6	6
15	6/4/36	1.80	1.80	1.85	1.95	20.0	19.5	—	—	—	+	—	—	—	+	—	—	+	—	+	—	4	8
16	6/4/36	1.80	1.81	1.86	1.90	22.0	21.2	—	—	—	+	—	+	—	+	—	—		—	—	—	3	8
17	6/4/36	1.80	1.82	1.86	1.85	24.0	23.0	—	—	—	—	+	—	—	—	—	+	—	—	—	—	2	10
7	5/16/36	2.00	2.04	2.10	1.99	16.0	16.0	—	—	+	—	+	+	+	+	—	+	+	+	+	+	10	2
8	5/16/36	2.00	2.08	2.05	1.95	18.0	18.0	+	+	—	+	+	+	+	+	+	+	+	—	+	—	9	3
9	5/16/36	2.00	2.09	2.04	1.98	20.0	20.0	+	—	+	+	+	+	+	—	+	+	+	+	+	—	10	2
10	5/16/36	2.00	2.00	2.00	1.95	22.0	22.0	—	—	—	—	—	—	—	—	—	—		—	+	+	2	9
11	5/16/36	2.00	2.06	2.05	1.85	24.0	24.0	—	—	—	+	—	+	—	+	+	+	+	+	+	+	9	3
84	4/18/36	2.20	2.23	2.15	1.90	14.0	13.8	+	+	+	+	+	+	+	+	+	+	—	+	—	+	10	2
85	4/18/36	2.20	2.20	2.17	1.85	16.0	16.0	+	+	+	+	+	+	+	+	+	+	—	+	+	—	10	2
86	4/18/36	2.20	2.20	2.14	1.85	18.0	17.0	+	+	+	—	+	+	—	+	+	—	+	+	+	+	9	3
87	4/18/36	2.20	2.17	2.20	1.78	20.0	19.5	+	+	—	+	+	+	+	+	+	+		+	+	+	10	1
88	4/18/36	2.20	2.25	2.10	1.78	22.0	21.5	+	+	—	+	+		+	+	+	+	+		—	—	7	3
89	4/18/36	2.20	2.20	2.13	1.78	24.0	24.0	—	+	—	—	+	—	+	—	+	+	—	—	+	—	5	7
																				Total.....		213	98
																				Per cent.....		68.5	31.5

The influence of sucrose, with a combination of acids (Table 3), on the viability of yeasts shows a preserving action as the concentration of sugar is increased from 14 Baumé to 24 Baumé. The decrease in the number of jars showing viable yeast is not as great as with acetic acid alone but is greater than with lactic acid alone, the percentage decrease in each case being 92 per cent for acetic acid, 28 per cent for lactic acid, and 58 per cent for a combination of the two acids.

In conclusion one more significant difference should be noted. In the acetic acid series (Table 1), 9.3 per cent of the jars showed viable yeasts, whereas in the lactic acid series (Table 2) 68.5 per cent, and with a combination of the two acids (Table 3) 42.4 per cent showed viable yeasts. Furthermore, with acetic acid the number of jars showing viable yeast at the end of the first week was 22.4 per cent and at the end of 20 weeks only 5.2 per cent; with lactic acid there was 57.4 per cent at the end of one week and still 57.4 per cent at the end of 20 weeks; while with a combination of the two acids there was 73 per cent at the end of the first week but only 13.5 per cent at the end of 20 weeks. Likewise the contamination in the jars containing lactic acid was considerably greater before inoculation than in the jars containing the combination of the two acids.

PICKLES EXPOSED TO AIR CONTAMINATION

At the end of 20 weeks the tops of all jars were taken off and the contents thus exposed to air contamination for two weeks. At the end of this time the loss of water by evaporation was replaced and the jars observed for several months. A violent fermentation took place in some of the jars so treated, and yeasts were isolated from them in large numbers. The acidities and Baumé of the jars in which fermentation took place are shown (Table 4).

COMBINATION NECESSARY FOR PRESERVATION

From the information obtained (Tables 1, 2, 3, and 4) it would seem that too great a percentage of the cans containing lactic acid had viable yeasts present at the end of the 20-week period to indicate a high degree of preserving action for this acid. This fact was demonstrated further when the cans were subjected to air contamination.

The lower concentrations of sugar are likewise conducive to yeast growth as indicated by the tables, especially at the lower acid concentrations. Even acetic acid was not a satisfactory preservative in the concentrations used when the sugar content was below 14 degrees

TABLE 3

Preserving Value of Various Percentages of Acetic and Lactic Acids With Sucrose for Pickles Inoculated With an Aciduric Yeast

No.	Date canned	Calculated values (per cent)			Actual values (per cent) acetic acid		pH 10/11/36	Baumé readings (degrees)		Test for presence of yeast							No. +	No. —
										Before inoculation	Time after inoculation								
		Acetic acid	Lactic acid as acetic acid	Total acidity as acetic acid	When canned	10/11/36		Calculated	Actual when canned		1 week	2 weeks	4 weeks	7 weeks	14 weeks	20 weeks		
54	9/22/36	1.20	.80	2.00	1.94	1.79	2.24	14.0	15.3	— —	+ +	— +	— +	— —	— —	— —	4	8
55	9/22/36	1.20	.80	2.00	2.05	1.91	2.16	16.0	16.9	— —	— —	— +	+ +	+ —	+ +	+ —	9	3
56	9/22/36	1.20	.80	2.00	2.03	1.89	2.12	18.0	18.9	— —	+ +	+ +	+ +	+ +	+ +	+ —	11	1
57	9/22/36	1.20	.80	2.00	2.00	1.92	2.08	20.0	20.3	— —	— +	+ +	+ —	+ +	— +	— —	7	5
58	9/22/36	1.20	.80	2.00	1.98	1.90	2.03	22.0	22.3	— —	+ +	+ +	— +	— +	— +	— —	7	5
59	9/22/36	1.20	.80	2.00	1.97	1.90	2.03	24.0	23.9	— —	+ +	+ +	+ —	+ —	— —	— —	6	6
73	9/22/36	1.40	.40	1.80	1.79	1.52	2.63	14.0	14.1	+ —	+ +	+ +	+ —	— —	— —	— —	5	7
74	9/22/36	1.40	.40	1.80	1.81	1.71	2.50	16.0	16.2	— —	+ +	+ +	— +	— —	— —	— —	5	7
76	9/22/36	1.40	.40	1.80	1.82	1.67	2.50	18.0	18.0	— —	+ +	+ +	— —	— —	—	— —	4	7
77	9/22/36	1.40	.40	1.80	1.79	1.63	2.29	20.0	20.1	— —	+ +	+	+ —	— —	— —	— —	4	7
42	8/20/36	1.40	.60	2.00	1.99	1.84	2.37	14.0	14.0	— —	+ +	+ +	+ +	+ +	— +	— +	10	2
43	8/20/36	1.40	.60	2.00	2.00	1.89	2.33	16.0	16.0	— —	+ +	+ +	+ +	+ +	— +	— —	9	3
44	8/20/36	1.40	.60	2.00	2.00	1.83	2.29	18.0	18.0	— —	+ +	— +	— +	+ +	— +	— —	7	5
45	8/20/36	1.40	.60	2.00	2.00	1.88	2.37	20.0	20.0	— —	+ +	+ —	+ +	— +	— —	+ —	7	5
46	8/20/36	1.40	.60	2.00	2.00	1.86	2.20	22.0	22.0	— —	— —	— —	— —	— —	— —	— —	0	12
47	8/20/36	1.40	.60	2.00	2.00	.....	2.29	24.0	.....	— —	— —	— —	— —	— —	— —	— —	0	12
48	9/22/36	1.60	.20	1.80	1.75	1.76	2.54	14.0	14.5	— —	+ +	+ +	— —	— —	— —	— —	4	8
49	9/22/36	1.60	.20	1.80	1.86	1.78	2.50	16.0	16.4	— —	+ +	+ +	— —	— —	— —	— —	4	8
50	9/22/36	1.60	.20	1.80	1.80	1.60	2.46	18.0	18.4	— +	+ +	+ +	— +	— —	— —	— +	6	6
51	9/22/36	1.60	.20	1.80	1.75	1.61	2.50	20.0	20.1	— —	+ +	+ +	+ +	— —	— —	— —	6	6
52	9/22/36	1.60	.20	1.80	1.80	1.59	2.37	22.0	22.0	— —	+ +	+ +	— —	— —	—	— —	4	7
53	9/22/36	1.60	.20	1.80	1.78	1.60	2.29	24.0	24.1	— —	+ +	+ +	— —	— —	— —	— —	4	7
18	7/11/36	1.60	.40	2.00	1.94	1.96	2.41	14.0	14.0	— —	— —	— —	— —	+ +	—	— +	3	8
19	7/11/36	1.60	.40	2.00	1.98	1.95	2.43	16.0	16.0	— —	— +	+ +	+ —	+ +	+ +	+ —	9	3
20	7/11/36	1.60	.40	2.00	2.02	2.05	2.37	18.0	18.8	— —	+ —	+ —	— —	— —	+ —	— —	3	9
21	7/11/36	1.60	.40	2.00	1.92	1.92	2.40	20.0	20.2	— —	+ —	— +	+ —	— +	+ —	— +	6	6
22	7/11/36	1.60	.40	2.00	1.93	1.86	2.31	22.0	22.2	— —	— +	— —	— +	— —	— —	— —	2	10
23	7/11/36	1.60	.40	2.00	2.10	1.98	2.29	24.0	25.0	— —	+ —	+ —	— —	+ —	— —	— —	3	9
24	7/11/36	1.80	.20	2.00	1.95	1.98	2.54	14.0	14.0	— —	— +	+ —	— —	+ +	— +	— —	5	7
25	7/11/36	1.80	.20	2.00	1.90	1.91	2.54	16.0	16.0	— —	+ —	+ —	+ +	+ +	+ +	— +	9	3
27	7/11/36	1.80	.20	2.00	2.06	2.03	2.46	20.0	20.7	— —	+ —	— —	— —	— —	— —	— —	1	11
28	7/11/36	1.80	.20	2.00	2.06	1.83	2.45	22.0	22.3	— —	— +	— +	— —	— —	— —	— —	2	10
29	7/11/36	1.80	.20	2.00	2.00	1.85	2.45	24.0	24.4	— —	— —	— —	— —	— —	— —	— —	0	12
																Total	166	225
																Per cent	42.4	52.6

Baumé. In a previous paper, Fabian (1934) pointed out that pickle manufacturers in actual practice observed that when the acidity went below 1.9 per cent they were approaching the danger point, especially in low Baumé pickles. These results confirm the experience of practice, since spoilage occurred at two per cent acetic acid at a 14-degree Baumé.

These results likewise demonstrate that it is not feasible to use lactic acid at low sugar concentrations. No spoilage occurred in any of the cans where a combination of acetic and lactic acids was used (Table 4). Certainly a Baumé of greater than 14 degrees is indicated and an acidity of not less than 1.8 per cent at the higher Baumé. In view of these findings, an acidity of not less than two per cent is

TABLE 4

Spoilage Owing to Air Contamination

Jar No.	Kind of acid	Acid as acetic	Baumé degrees	Jar No.	Kind of acid	Acid as acetic	Baumé degrees
		pct.				*pct.*	
1	Lactic	1.4	14	12	Lactic	1.8	14
2	Lactic	1.4	16	13	Lactic	1.8	16
30	Lactic	1.6	14	7	Lactic	2.0	16
31	Lactic	1.6	16	8	Lactic	2.0	18
32	Lactic	1.6	18	60	Acetic	2.0	14
36	Acetic	1.6	14	84	Lactic	2.2	14

always recommended in order to be absolutely sure of the product being preserved under all conditions.

GENERAL DISCUSSION

On an equal-acidity basis the results show that lactic acid is not so good a preservative as acetic acid. This confirms the results of Fabian and Johnson (1938) who found that it required .3 per cent lactic, calculated as acetic, but only .2 per cent acetic acid, to inhibit the growth of sporogenic bacteria, such as *Bacillus mesentericus fuscus*. These results are contrary, however, to the findings of Iszard (1927) who found that lactic acid was superior to acetic acid for controlling the growth of *Bacillus patasites* isolated from mayonnaise.

These experiments also demonstrated that under the conditions present pH was not a reliable indicator of the preserving value of an acid. At the same concentrations the pH for lactic acid was lower than for acetic acid, yet the preserving power of the latter was greater than the former. Likewise, when a combination of the two acids was used, the preserving power of the acid solution proportional to the amount of acetic acid present.

SUMMARY AND CONCLUSIONS

When the acetic acid was kept constant and the concentration of sucrose was varied from 14 to 24 Baumé for each series of experiments, there was a gradual decrease in the number of jars of pickles showing viable yeasts as the percentage of acid was increased by increments of .2 per cent for each series. However, there was no material decrease beyond two per cent acetic acid.

When the amount of sucrose was kept constant and the percentage of acetic acid varied, there was a gradual reduction in the number of jars showing viable yeasts. No significant reduction occurred, however, until a 22 Baumé had been reached.

Under the same conditions, increasing the lactic acid content by increments of .2 per cent did not produce nearly so great a reduction in the number of jars showing the presence of viable yeasts as acetic acid did in the presence of the same sugar concentration—this despite the fact that the lactic acid solutions had a lower pH value. Likewise, increasing the sugar concentration in the presence of lactic acid did not reduce the number of jars showing viable yeasts nearly so much as did acetic acid.

In a similar series of experiments using a combination of acetic and lactic acids in the presence of varying amounts of sucrose, the preserving action was proportional to the amount of acetic acid used and occupied an intermediate position between acetic and lactic acids. The preserving action was less than when acetic acid was used alone but greater than with lactic acid alone. The influence of increasing the concentration of sucrose on the viability of yeasts likewise is not so great in a combination of the two acids as with acetic acid alone but greater than with lactic acid alone.

An acidity of not less than two per cent acetic or a combination of acetic and lactic acids in the ratios used in these experiments is recommended for preserving pickles or pickle products.

Under the conditions of these experiments, pH is not a reliable indicator of the preserving power of the acids present.

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EXPERIMENTAL WORK ON LACTIC ACID IN PRESERVING PICKLES AND PICKLE PRODUCTS

III. USE OF LACTIC ACID IN PICKLES AND RELATED PRODUCTS [1]

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In judging the flavor of sweet pickles, relish, and other pickle products several factors enter into consideration in determining the consumer preference of the product. The first consideration is the differences in the individual tastes of various persons. Some like sweet and some like sour pickles to a varying degree. Second, there are the different materials in the pickle itself, such as sugar, salt, acid, and spices. All play a very important part in determining the final flavor of the pickle. Too high an acidity in the presence of a low sugar concentration gives a sour taste, while too much spicing gives a "cutting" sensation, especially in the throat after the pickle has been swallowed. Salt in pickles, as in any other food, plays a very important part in flavoring which is not appreciated by some manufacturers.

An ideal pickle flavor is one that does not leave the person eating it conscious of any one taste, such as sweet, sour, spicy, or salty. It is a complete blending of all the individual flavors into a harmonious whole in which no one flavor is predominant or perceptibly discernible above any of the others.

In testing the pickles made in these experiments a standard was made to guide the tasting in so far as possible. An outline of these terms under the various headings (Table 1) is self-explanatory for the most part except in the case of the terms under "spicing"; "very spicy" indicates a predominating sense of spices to the exclusion of other flavors. The sensation is one of "bitiness" and leaves a "cutting" sensation when the pickle is swallowed. "Good balance" means that no one spice in the mixture is perceptible above any other one. The proportion of each spice in the mixture is such that the flavor of any one spice, such as cloves or cinnamon, does not predominate. Under this heading are also listed the sub-headings "excessive," which means that although the mixture of spices is well

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balanced, the spicy flavor is noticeable above the other flavors and may leave a "biting" sensation on the tongue or the "cutting" sensation in the throat but to a lesser degree than in the case of "very spicy." "Good" indicates a well-blended flavor of spices with the acidity, saccharinity, and salinity of the pickle. It is "good" both as regards the blending of the spices so that no one flavor of spice is predominant and also as regards the amount present in the pickles. It is the ideal to attain in spicing. "Insufficient" means that the spicing is barely perceptible. "Poor balance" means some one flavor of spice is perceptible above the blend of the others so that one flavor

TABLE 1

Suggested Standard Terms for Organoleptic Tests for Sweet Pickles and Relish

I. Acidity	II. Sweetness	III. Spicing
1. Sharp	1. Very sweet	1. Very spicy
2. Very sour	2. Sweet	2. Gool balance
3. Sour	3. Mildly sweet	(a) Excessive
4. Sweet sour	4. Sweet sour	(b) Good
5. Mild	5. Sour	(c) Insufficient
	6. Very sour	3. Poor balance
	7. Sharp	(a) Cinnamon taste predominates
		(b) Clove taste predominates
IV. Salinity	V. General Conclusions	(c) Other spices predominate
1. Very salty	1. Excellent	
2. Noticeable	2. Good	
3. Absent	3. Fair	
	4. Poor	
	5. Bad	

predominates, such as mustard, cloves, ginger, or cinnamon. Since some spicing mixtures contain as many as 20 different spices, the blending of them is very important; and when a good balance is obtained, it is carefully guarded by the manufacturer wishing to produce that particular well-balanced flavor in his product.

USE OF LACTIC ACID IN SOUR PICKLES

Experiments were conducted using lactic acid alone and in combination with acetic acid for making sour pickles. Lactic acid, when used alone, made the pickles too sour in the concentrations necessary to prevent spoilage. Likewise, as previous experiments by Fabian and Wadsworth (1939) had shown, the pickles soon became soft. When used in conjunction with acetic acid, lactic acid in small amounts did not appreciably affect the flavor, and it was impractical

to add too much since it detracted from the keeping quality of the pickles. For these reasons this series of experiments was discontinued with the conclusion that it was not feasible to use lactic acid for making sour pickles.

USE OF LACTIC ACID IN RELISH

Relish was made according to an approved formula of a large manufacturer. The relish was preserved at different degrees Baumé ranging from 18 to 24 at two-degree intervals with different combinations of acetic and lactic acids (Table 2).

The relish was plated on malt extract agar, glucose agar, and glucose agar acidified to one per cent with vinegar. No growth occurred in any of the plates. Inoculations were made from the relish into tubes of malt extract broth, glucose broth, and glucose broth acidified to .5 per cent with vinegar. Some of the tubes of malt extract and glucose broth, but none of the tubes of acidified broth, developed a scum on the surface (Table 2). When this scum was inoculated into the broth containing .5 per cent acid, no growth occurred. A microscopic examination of the scum showed it to be produced by a sporulating rod. Evidently some samples of the relish contained an aerobic, sporogenic, rod-shaped bacterium which had survived the preservatives. Since it did not grow in acid broth, no significance was attached to it.

USE OF LACTIC ACID IN SWEET PICKLES

Several lots of sweet pickles were made with cider vinegar at different acidities and at varying degrees of Baumé for comparison as to the physical condition of the pickles, such as crispness, color, flavor, with those made from a combination of acetic and lactic acids. In the experiments (Tables 3 and 4) the spicing was the same, so that no variable would be introduced from it. The method of spicing was one recommended by a large manufacturer.

One man made all the tests recorded (Table 3) and according to his judgment there were but two "good" batches of pickles and they were the ones having 1.8 per cent acetic acid with an 18-degree Baumé and 1.6 per cent acetic acid with a 24-degree Baumé.

The results of making pickles with a combination of acetic and lactic acids at different degrees Baumé (Table 4) show that with these combinations many more samples were judged good and one was judged excellent. The chief objection to these pickles was the condition of them. They did not seem to be so firm on short standing, in most cases, as those made with acetic acid. Later they became very firm and crisp and are still good after nearly three years.

TABLE 2

Combinations of Acetic and Lactic Acids at Different Degrees Baumé Used in Making Relish

No.	Degrees Baumé	Acetic acid	Lactic acid calculated as acetic acid	Total acidity calculated as acetic	Growth on agar			Growth in broth			Remarks on flavor
					Malt extract	Glucose	Glucose +1% H Ac.	Malt extract	Glucose	Glucose +.5% H Ac.	
		pct.	*pct.*								
1	18	1.6	.4	2.0	—	—	—	—	—	—	Fair
2	18	1.8	.2	2.0	—	—	—	—	—	—	Fair
3	18	2.0	.2	2.2	—	—	—	—	—	—	Good
4	18	2.0	.4	2.4	—	—	—	—	—	—	Good
5	18	2.2	.0	2.2	—	—	—	—	—	—	Fair
6	18	2.2	.2	2.4	—	+	—	+	+	—	Fair
7	20	1.6	.4	2.0	—	—	—	—	—	—	Good
8	20	1.8	.2	2.0	—	—	—	—	—	—	Good
9	20	2.0	.2	2.2	—	+	—	+	+	—	Fair
10	20	2.0	.4	2.4	—	—	—	—	—	—	Fair
11	20	2.2	.0	2.2	—	—	—	—	—	—	Fair
12	20	2.2	.2	2.4	—	—	—	—	—	—	Poor
13	22	1.6	.4	2.0	—	—	—	—	—	—	Good
14	22	1.8	.2	2.0	—	—	—	—	—	—	Fair
15	22	2.0	.2	2.2	—	—	—	—	—	—	Fair
16	22	2.0	.4	2.4	—	—	—	—	—	—	Good
17	22	2.2	.0	2.2	—	—	—	—	—	—	Fair
18	22	2.2	.2	2.4	—	—	—	—	—	—	Good
19	24	1.6	.4	2.0	—	+	—	+	+	—	Excellent
20	24	1.8	.2	2.0	—	—	—	—	—	—	Good
21	24	2.0	.2	2.2	—	—	—	—	—	—	Good
22	24	2.0	.4	2.4	—	—	—	—	—	—	Good
23	24	2.2	.0	2.2	—	—	—	—	—	—	Fair
24	24	2.2	.2	2.4	—		—	—	—	—	Good

TABLE 3

Observations on Sweet Pickles With Vinegar Alone

Baumé	Acetic acid	Condition of pickle	Color of pickle	Acidity	Sweetness	General conclusions as to quality
	pct.					
14	1.8	Firm	Pale green	Very sour	Sweet sour	Fair
16	1.8	Crisp	Some pale green; others showed some loss of green	Very sour	Sweet sour	Fair
18	1.8	Firm; fairly crisp	.....	Mild	Mildly sweet	Poor
20	1.8	Firm and crisp	Partly green	Mild	Mildly sweet	Good
22	1.8	Firm but not crisp	Uniformly green	Mild	Very sweet	Fair
24	1.8	Firm but not crisp	Uniformly green	Mild	Very sweet	Fair
18	2.0	Firm and crisp	Pale green	Mild	Very sweet	Fair
20	2.0	Firm and crisp	Pale green	Mild	Sweet	Poor
22	2.0	Firm but not crisp	Pale green	Mild	Sweet sour	Fair
24	2.0	Firm but not crisp	Pale green	Mild	Sweet	Fair
14	2.2	Firm	Pale green	Sour	Sour	Poor
18	2.2	Firm and crisp	Green, uniformly	Sour	Mildly sweet	Fair
22	2.2	Firm but not crisp	Brown center	Mild	Sweet	Fair
24	2.2	Firm and crisp	Slightly green	Mild	Sweet	Fair
18	2.4	Firm and fairly crisp	Very good	Sour	Sweet sour	Fair
24	2.4	Not very firm; fairly crisp	Little too green	Sweet sour	Very sweet	Fair
18	1.6	Firm; not crisp	Good	Mild	Sweet	Fair
24	1.6	Very firm; very crisp	Pale green	Mild	Mildly sweet	Good

TABLE 4

Observations on Sweet Pickles Made With a Combination of Vinegar and Lactic Acid

Baumé	Acetic acid	Lactic as acetic acid	Total acidity as acetic	Condition of pickle	Color of pickle	Acidity	Sweetness	General conclusions as to quality
	pct.	*pct.*						
16	1.4	.4	1.8	Firm, but slightly rubbery	Uniformly green	Mild	Very sweet	Fair
18	1.4	.4	1.8	Firm, but slightly rubbery	Uniformly green	Sweet sour	Very sweet	Fair
22	1.4	.4	1.8	Rubbery	Variable	Mild	Very sweet	Fair
24	1.4	.4	1.8	Rubbery	Pale green	Mild	Mildly sweet	Good
20	1.4	.6	2.0	Rubbery	Uniformly green	Mild	Very sweet	Poor
14	1.6	.2	1.8	Firm, yet rubbery; not crisp	Uniformly green	Sour	Sour	Poor
16	1.6	.2	1.8	Firm, yet rubbery; not crisp	Uniformly green	Sour	Sour	Poor
18	1.6	.2	1.8	Fairly firm	Uniformly green	Mild	Very sweet	Fair
20	1.6	.2	1.8	Firm, yet rubbery	Pale green	Mild	Very sweet	Fair
24	1.6	.2	1.8	Rubbery	Pale green	Mild	Sweet	Poor
14	1.6	.4	2.0	Firm, but slightly rubbery	Uniformly green	Sweet sour	Sweet sour	Good
16	1.6	.4	2.0	Fairly firm, but slightly rubbery	Uniformly green	Sweet sour	Sweet sour	Good
18	1.6	.4	2.0	Not very firm	Uniformly green	Sour	Mildly sweet	Good
22	1.6	.4	2.0	Slightly rubbery; not crisp	Uniformly green	Mild	Sweet	Poor
24	1.6	.4	2.0	Crisp; firm	Uniformly green	Mild	Mildly sweet	Excellent [1]
14	1.8	.2	2.0	Very firm; very crisp	Uniformly green	Very sour	Sweet sour	Fair
16	1.8	.2	2.0	Rubbery; not crisp	Uniformly green	Very sour	Sweet sour	Fair
20	1.8	.2	2.0	Firm; not crisp	Pale green	Mild	Mildly sweet	Good
22	1.8	.2	2.0	Firm; very crisp	Uniformly green	Mild	Very sweet	Good

[1] Best of all.

USE OF LACTIC ACID IN PROCESSED DILLS

As previously stated, the ratio of lactic to acetic acid in genuine dill pickles is 8 : 1. It is, therefore, logical to expect that the addition of lactic acid to processed dills would greatly improve their flavor. The experiments (Table 5) support this supposition. The greatest improvement in flavor seemed to be in the pickles made with lactic acid and distilled or white vinegar. Here there were three combinations that gave very good results and were a decided improvement upon acetic acid alone. The spicing and salting were

TABLE 5

Observations on Processed Dill Pickles Made With a Combination of Acetic and Lactic Acids

Lactic acid as acetic	Acetic acid	Source of acetic acid = white vinegar			Source of acetic acid = cider vinegar		
		Acid	Salt	General opinion	Acid	Salt	General opinion
pct.	*pct.*						
1.0	0.0	Sour	Noticeable	Fair	Sour	Noticeable	Fair
0.8	0.2	Very sour	Noticeable	Fair	Sour	Noticeable	Fair
0.6	0.4	Sour	Noticeable	Bad	Sour	Noticeable	Fair
0.4	0.6	Sour	Noticeable	Bad	Sour	Noticeable	Good
0.2	0.8	Sour	Noticeable	Poor	Sour	Noticeable	Good
1.2	0.0	Sour	Noticeable	Bad	Sour	Noticeable	Fair
1.0	0.2	Sour	Noticeable	Excellent	Sour	Noticeable	Fair
0.8	0.4	Sour	Noticeable	Excellent	Sour	Noticeable	Excellent [1]
0.6	0.6	Sour	Noticeable	Excellent	Sour	Noticeable	Good
0.4	0.8	Sour	Noticeable	Fair	Sour	Noticeable	Poor
0.2	1.0	Sour	Noticeable	Poor	Sour	Noticeable	Poor
0.8	0.0	Sour	Noticeable	Poor	Sour	Noticeable	Poor
0.6	0.2	Sour	Noticeable	Poor	Sour	Noticeable	Poor
0.4	0.4	Sour	Noticeable	Fair	Sour	Noticeable	Poor
0.2	0.6	Sour	Noticeable	Fair	Sour	Noticeable	Poor

[1] Best of all.

kept constant for all lots of pickles so that the only variable was the acidity.

FLAVOR TESTS BY PICKLE MANUFACTURERS

At the annual Pickle Packers and Kraut Conference,[2] Wadsworth (1937) placed a large number of samples of pickles, which had been made from acetic acid and combinations of acetic and lactic acids, before experts in pickle manufacturing. All samples were given numbers so as to remove all prejudices and knowledge of the product being tasted so that the pickles were judged solely on their merits (Table 6).

[2] Held at East Lansing, February 24-26, 1937.

Organoleptic tests on Samples A and B, identical in every respect except the acids, gave a 70-per cent preference for the combination of acetic and lactic acids as compared with acetic alone. With Samples C and D the ratio was 65 per cent preference in favor of acetic acid. A combination of 1.6 per cent acetic to .4 lactic acid was compared with 1.8 per cent acetic to .2 per cent lactic acid at 24 degrees Baumé and gave an 85-per cent preference in favor of the latter. In Samples G and H at 22 degrees Baumé, the combination of 1.8 per cent acetic to .2 per cent lactic again showed to advantage since

TABLE 6

Results of Judgment of a Group of Pickle Manufacturers on Flavor of Sweet and Processed Dill Pickles

Sample	Baumé	Acetic acid	Lactic acid	Total number samples tasted	Preference
		SWEET PICKLES			
		pct.	*pct.*		*pct.*
A	24	1.8	0.2	26	70
B	24	2.0	0.0	26	30
C	20	2.0	0.0	23	65
D	20	1.8	0.2	23	35
E	24	1.6	0.4	20	15
F	24	1.8	0.2	20	85
G	22	1.8	0.2	21	76
H	22	2.0	0.0	21	24
		PROCESSED DILL PICKLES			
1	....	1.0	0.2	18	67
2	....	0.6	0.6	18	33
3	....	0.4	0.8	11	46
4	....	0.8	0.4	11	54

there was a 76-per cent preference for the combination compared with a 24-per cent preference for acetic acid alone.

From these results it is obvious that the flavor of sweet pickles is improved by the addition of lactic acid to acetic acid in the finishing process. The ratio to use depends upon several factors: first, the amount of sugar present; second, the amount of spicing used; and third, the consumer preference. The suggested ratios to use in percentages of acetic and lactic (expressed as acetic) acids, respectively, are 1.6 : .4; 1.7 : .3; or 1.8 : .2.

Likewise, lactic acid has a real use in making processed dill pickles. Through an error on our part no processed dill pickles made with acetic alone were placed out to sample in comparison with those made from the various combinations of acetic and lactic acids. From

our own observations (Table 5), however, and those of others who sampled the processed dill pickles made with acetic acid alone and combinations of the two acids, there is no question but that the flavor is improved by the addition of lactic acid to acetic acid in the ratio of .2 : 1; .4 : .8, or even as high as .6 : .6 per cent, respectively, in the case of distilled vinegar. Detailed instructions as to cost and how to use lactic acid in combination with acetic acid has been published elsewhere by Wadsworth and Fabian (1939).

CONCLUSIONS

Lactic acid is not suitable for making sour pickles either from the standpoint of flavor or keeping properties when used alone or in combination with acetic acid.

Lactic acid is not suitable for making sweet pickles or relish when used alone, but greatly improves the flavor of these products when used in the proper ratios with acetic acid. The best ratios according to our results were 1.6 : .4 or 1.8 : .2 per cent of acetic and lactic acids, respectively.

A combination of lactic and acetic acids gave better-flavored processed dill pickles than either acid alone. The ratio of acetic to lactic calculated as acetic which gave the best results was 1 : .2; .8 : .4, or even as high as .6 : .6 per cent, respectively, with distilled vinegar.

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EDITORIAL REVIEW

USES AND LIMITATIONS OF THE COLIFORM GROUP IN SANITARY CONTROL OF FOOD PRODUCTION[1]

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The volume of literature published on the coliform[2] bacteria, their identification, differentiation, and ecological relationships in connection with sanitation, attest to the importance of this group of organisms in the field of applied bacteriology. Because of the generally recognized normal habitat of certain members of the group it has been, almost from the beginning of the science of bacteriology, and still is, the practice to use coliform organisms as measures of pollution of food and water. In the sanitary control of food production and handling there is, of necessity, considerable groping for bacteriological indices of pollution and potential danger to health which presents to the bacteriologist who is called upon to apply control measures the problem of how to interpret the presence of coliform bacteria, not only as a group but when identified as to type. Continuing researches with this group of organisms and the development of a multiplicity of differential tests brought about a trend of thought which, in quite recent years, resulted in splitting the group into a bewildering number of species and varieties. The action recently taken by those who have compiled the fifth edition of Bergey's *Manual of Determinative Bacteriology* (1939) in simplifying the nomenclature of the coliform organisms marks a definite and welcome step toward the correction of a condition which could give rise only to confusion in the minds of those who deal with the applied aspects of the problem.

Parr (1939), in his recently published monograph, has presented an excellent account of the development of the present classification and characterization of the coliform bacteria. To the bacteriologist exerting control over sanitation there may be some uncertainty as to

[1] Presented at the Third International Congress for Microbiology, September 5, 1939, at New York City.

[2] Throughout this discussion the writer has followed the recommendation of Breed and Norton (Am. Jour. Pub. Health **27**, No. 6 [1937] 560-563) in using the term "coliform" to designate lactose-fermenting, aerobic, non-sporulating organisms comprising the group often referred to as "colon-aerogenes" or "Escherichia-Aerobacter" group.

the position in which he is placed by the results of those investigations which emphasize the instability of differential characteristics and the intergradations between species. The well-recognized variations occurring among coliform bacteria, however, need not, in our opinion, invalidate the usefulness of the group as a measure of insanitary conditions surrounding food production, provided ample information regarding the significance of coliform organisms in any given type of food product is at hand to enable a proper interpretation of their presence. It is apparently the consensus of opinion that those coliform organisms which are indol positive, methyl-red positive, Voges-Proskauer negative, and do not utilize citric acid as a source of carbon (so-called Imvic + + — —) are useful as a measure of excretal contamination. On the other hand, regardless of the fact that they do occur in feces and are included in the *B. coli* group as a basis of condemnation of some commodities, coliform organisms now included in the genus *Aerobacter* which are indol negative, methyl-red negative, Voges-Proskauer positive, and citrate positive (Imvic — — + +) are not regarded, except in special instances, as having sufficient sanitary significance to be of definite value as indices of pollution. The ground for uncertainty lies with the so-called intermediates now classified as *Escherichia freundii*. This uncertainty has long existed, but concepts arrived at from recent publications in this country do little to disturb the theory that intermediates occurring in some food products may have sanitary significance. In fact, Parr (1938a) has stated that they do occur in human feces to such degree that they must be considered, for purposes of sanitary control, as indices of excretal contamination. While we can concur with this conclusion and under certain circumstances advocate its application, a program of caution, where the burden of proof beyond a reasonable doubt must be assumed, dictates serious consideration of those points brought out by Parr (1939, 1938b); Stuart, Griffin, and Baker (1938); and by others whose reports are cited by these writers, regarding the instability of differential characteristics by which the intermediates are identified and concerning the failure of investigators as yet to demonstrate that certain intermediates are peculiar to a given environment.

The history of sanitary control of water, oysters, and milk has shown that the sanitary significance of coliform bacteria in these products must be evaluated in the light of knowledge available concerning the source of the commodity and the methods of its preparation and handling. A knowledge of the food product which permits an explanation of the sources and routes of contamination is fully as important as a knowledge of the identity of the coliform

organisms detected in or on it. This principle is elementary, so much so that it would appear unnecessary to state it here; but since, in the conduct of sanitary-control programs, it is not unusual to encounter misconceptions and differences of opinion relative to the applicability of coliform bacteria as indices of pollution of food, it seems desirable to clarify a viewpoint which should, and ordinarily does, prevail. Determination of the presence of coliform organisms does serve a very useful purpose when there has been sufficient study of the methods of production and handling of the class of food product under control to lay the basis for interpretation of the significance of members of the coliform group as a whole or of genera or species within the group. Until this basis is laid the very ubiquity of the group places a handicap upon the interpretation of results obtained by laboratory examination.

To illustrate this point attention is called to the fact that in the sanitary control of drinking water the bacteriological standard adopted by the United States Treasury Department (1925) is expressed in terms of the coliform group as a whole. Moreover, on the authority of "Standard Methods" (1936) any attempt to evaluate the sanitary quality of drinking water on the basis of a distinction between genera or species of coliform bacteria is unwarranted. In recognition of the weight to be given to survey data bearing upon the source of the water and to other factors affecting its sanitary quality, however, the committee appointed by the Surgeon General to formulate standards saw fit to recommend that the bacteriological standard be applied with some latitude; in other words that laboratory findings should be evaluated in the light of all other available evidence.

The system of control established by the United States Public Health Service (1937) for the guidance of local agencies responsible for supervision over sanitation of oyster production provides for the classification of oyster-growing areas according to sanitary survey data supplemented by bacteriological examinations for the detection of the coliform group in the sea water, Old (1937). In these examinations no differentiation is made of bacteria within the group. Among those who have an intimate interest in this field of applied bacteriology there is no unanimity as to whether the coliform group as a whole or *Escherichia coli* should be used as a measure of pollution, Perry (1937). Perry and Bayliss (1936) have emphasized the lack of correlation between degrees of pollution of growing areas and the incidence of the coliform group as a whole. Furthermore, Perry (1936) and Perry and Hajna (1935) have recommended that in judging the sanitary quality of oysters the concentration of

Escherichia coli rather than that of the coliform group should be the guide. The desirability of ascertaining the numbers of *Escherichia coli* has also been pointed out by Hunter (1936). In general, those who dissent from Perry's recommendation as to differentiation of coliform bacteria appear to have to deal with oysters produced in geographical areas where, during the warmer seasons, organisms of the genus *Aerobacter* do not constitute the problem that they do for the control bacteriologist in other areas. Regardless of the outcome of the controversy as to whether the coliform group in its entirety or only a portion of it should be used as an index of pollution in oyster control, and whether in the final analysis this may be a matter for local determination, it is pertinent, in line with our theme, to direct attention again to the fact that the control system eventually adopted is followed because of a full knowledge of the sources and routes of contamination.

Because of knowledge acquired concerning means of their ingress into milk and their behavior in that product, coliform bacteria in high-count raw milk fade into insignificance as a measure of insanitary production. On the other hand, as the result of correlative investigations their presence in pasteurized milk can be accepted as evidence of faulty processing or of recontamination. Bartram and Black (1937a, 1937b) report that the presence of coli-aerogenes organisms in one cubic centimeter of pasteurized milk in 10 to 20 per cent of the samples is sufficient to indicate contamination and that the *Escherichia-Aerobacter* group as a whole is more significant than any one genus or species. Stuart, Wheeler, and Griffin (1938) and Wilson (1935) give special weight to a determination of the presence of *Escherichia* organisms, that is, those which according to the "Imvic" system are + + — —, as evidence of fecal pollution of milk. Stuart and his collaborators state that too much stress should not be placed upon the finding of the coliform group as a whole in certified milk and that in recognition of the sources of *Aerobacter* and intermediates in dairies differentiation of coliform types should be carried out in applying the standard of less than 10 coliform organisms per cubic centimeter approved for certified milk by the American Association of Medical Milk Commissions.

With the viewpoint so well illustrated by the principles followed in the sanitary control of water, oysters, and milk a similar approach has been made toward control of sanitation in the crabmeat industry by Hunter (1934) and Nickerson, Fitzgerald, and Messer (1939). Consideration of the methods of preparation and handling crabmeat clearly indicates the soundness of the conclusion that coliform bacteria, particularly those identified as *Escherichia coli*, have no proper

place in the product and may be accepted as indices of pollution and potential danger. In the preparation of crabmeat for the market the product is subjected to extensive handling after the crab, with its shell intact, has been cooked for sufficient time to destroy all vegetative forms of bacteria, if not to render the crab sterile. It naturally follows, therefore, that coliform bacteria present on the finished product must have gained access in the course of progress of the crabmeat through the plant. To anyone familiar with the process it is not difficult to appreciate the opportunities for contamination. In effecting control over the production of crabmeat it has been the practice to attach sanitary significance only to organisms identified as *Escherichia coli*, specifically to those which are indol positive, methyl-red positive, Voges-Proskauer negative, and citrate negative. The conclusion of Parr, previously cited, regarding intermediates as indicators of excretal contamination, indicates that this may be a lenient attitude. Experience and definitely planned investigations, however, have shown a correlation between the presence in crabmeat of *Escherichia coli* of the type just described and certain avenues of pollution with human waste. Some practical difficulties in carrying out a program of control have arisen from a lack of methods of rapid differentiation and from the instability of differential characteristics of the various types as well as from a lack of agreement among bacteriologists regarding the sanitary significance of *Aerobacter* and intermediates. Therefore, in using coliform bacteria as a measure of insanitary practices in the production of crabmeat the theory has been that all members of the coliform group are not necessarily of equal importance but that a knowledge of the probable sources of contamination fully justifies condemnation of the product when coliform bacteria, differentiated as + + — — in the "Imvic" system and hence predominatingly of intestinal origin, are found in the food. Plant inspection has invariably disclosed evidence of insanitary conditions or practices which are reflected in such bacteriological findings.

As a result of the practical uses made of coliform bacteria in the sanitary control of the products so far discussed a hope is formulated in the mind of the bacteriologist charged with the enforcement of sanitary measures that similar application can be made to other foods. The question arises as to how far efforts in this direction can be extended. Recently considerable attention has been directed to coliform bacteria occurring on shelled nut meats. Prucha (1936), Tracy and Brown (1936), Wenzel and Black (1939), and Ostrolenk and Hunter (1939) have submitted papers on this subject. The latter workers have proceeded far enough with investigations still

in progress to learn that it is not at all unusual to find *Escherichia coli* on shelled nut meats in spite of the fact that on nut meats removed aseptically from the shell there are no coliform organisms. There are clear indications that coliform bacteria present on shelled nut meats are closely correlated with improper conditions of handling and that further investigations may demonstrate definite channels of contamination to permit evaluation of laboratory findings in terms of insanitary methods of production or distribution.

Some attention has been given to the significance of coliform bacteria on fresh fish and fish fillets. Griffiths and Fuller (1936) have stated that *Escherichia coli* on fish fillets are indices of unclean methods since such organisms have no place there under normal conditions.

It is hardly necessary to point out that there are numerous food products, both simple and compound, in which there is no valid reason for the presence of coliform bacteria under normal conditions. On the other hand, we may recognize the limitations imposed upon an interpretation of the presence of coliform bacteria in products such as garden vegetables, eggs, and dairy products in general. We are in hearty accord with those who would decry the indiscriminate use of coliform organisms as indices of pollution or of potential danger wherever or whenever found on foods without some correlative data to establish the route of contamination, not necessarily on the individual sample, but on that class of food product prepared and handled under conditions that are recognized and understood. The coliform bacteria do represent the only bacterial group which at present can be used with even a reasonable degree of accuracy as a measure of pollution. With due regard to the distinct limitations imposed upon its use in the case of foods which may, by reason of conditions surrounding their production, normally contain coliform bacteria and in the case of food specimens selected at random for examination without an adequate background of information concerning production, we are disposed to believe that the usefulness of the coliform group can be extended to the sanitary control of a number of foods to which attention of this kind has not yet been given. While recently published reports lead to some discouragement of the hope that definite and unquestioned correlations may be established between species or varieties of coliform bacteria and a given environment, these reports appear not to discredit the theory that certain organisms of that group, namely, those of the species *Escherichia coli* which have the Imvic reactions + + — —, are directly or indirectly of fecal origin and that their presence in a food may be accepted as substantial evidence

of excretal contamination. Sound application of that theory may then be made with each new class of food product in the light of information obtained by thorough correlative studies conducted to establish a sound basis for conclusions as to the significance of coliform bacteria in terms of pollution during preparation or distribution.

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FUNGISTATIC PROPERTIES OF THE FATTY ACIDS AND POSSIBLE BIOCHEMICAL SIGNIFICANCE

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(Received for publication, April 3, 1939)

A study has been made to show the effect on growth of common molds when substances both fat and water soluble are introduced into the culture medium. Materials of this type were studied because of the well-known fact that many molds and particularly the spores contain considerable quantities of fat. Butyric acid, among the first of the substances tried, showed remarkable fungistatic properties. Substitution of salt in place of the acid indicated that the pH of the medium was an important factor to be considered.

A study of the fungistatic properties, over a wide pH range, of the following acids has been completed: all of the normal saturated fatty acids containing from one to 14 carbon atoms; isomeric forms, such as iso-butyric; the three isomeric forms of n-valeric; some of the unsaturated acids, such as crotonic and hendecenoic; and some of the substituted acids, such as β-iodopropionic.

Kiesel (1913) investigated the effect of a large number of organic acids on mold growth using Raulin's Solution as the nutrient medium. Since Raulin's Solution contains tartaric acid and has a pH of 2.8, his work applied only to conditions existing in highly acidic media. Rideal (1921) reported that formic acid is an effective mold inhibitor although this present study showed that formic acid has very limited fungistatic properties. Kirby, Atkin, and Frey (1937) described the fungistatic properties of acetic and other organic acids. On the other hand, Walker and Coppock (1928) showed that calcium propionate was utilized by mold as a source of carbon. The uncertainties, and in many cases contradictions, existing in this field have been excellently summarized by Bull (1937).

EXPERIMENTAL PROCEDURE

The fungistatic properties of the fatty acids were studied on an agar medium in Petri dishes, the medium being buffered at the desired pH with a suitable buffer mixture. The medium consisted of standard nutrient agar of one and one-half times normal strength with one per cent added sucrose. It was necessary, obviously, to use buffers which were non-toxic to molds. The buffers of McIlvaine (1921) were found satisfactory as neither citric acid nor disodium

phosphate had any effect on mold in the concentrations used. The buffer solutions were made up to twice the strength called for by McIlvaine; that is, .4 molar disodium phosphate and .2 molar citric acid were mixed in the proper proportions to give the desired pH value.

Fatty acid solutions were prepared in two-per cent and ten-per cent concentrations; for example, one gram of propionic acid was placed in a ten-ml. tube with five ml. of water and the proper indicator. The solution was then neutralized with ammonia water to the desired pH, and then made up to volume. The two-per cent solutions were prepared by diluting two ml. of the ten-per cent solution with eight ml. of distilled water. In the case of some of the higher acids, such as lauric, it was necessary to neutralize completely in order to obtain a clear solution.

TABLE 1

Concentration of Butyric Acid Necessary to Inhibit Mold at pH5

Desired concentration of butyric acid	2% butyric acid solution adjusted to pH 5	Buffer pH 5	Media	Mold growth in 48 hours at 37.5°C. (99.5°F.)
pct.	*ml.*	*ml.*	*ml.*	
.10	.50	4.50	5	+
.12	.60	4.40	5	+
.14	.70	4.30	5	0
.16	.80	4.20	5	0

In the preparation of a plate the correct amounts of buffer and fatty acid solution were measured into a Petri dish and well mixed by agitation. The melted agar was then added and the contents again well mixed. After the agar had set, the plate was seeded with a mixed mold culture. The method of seeding used was as follows: a spatula was dipped in the mold culture, held over the open Petri dish, and gently tapped. Sufficient spores were added to be visible when the dish was held over white paper. Care was taken to avoid clumping of spores. After seeding, the plate was incubated 48 hours at 37.5°C. (99.5°F.). Example of plating to show the concentration of butyric acid necessary to inhibit mold at pH 5 is shown (Table 1).

In the above example .14 per cent was taken as the critical concentration at a pH of 5. A plate is considered negative when no mold growth can be detected by the naked eye.

Since pH was such an important factor in this study, care was taken to obtain the desired pH on the finished plates. Numerous tests indicated that the buffer solutions were sufficiently strong to attain and to hold the desired pH. The pH values of the buffer solu-

tions, the fatty acid solutions, and the finished media were determined with standard LaMotte colorimetric equipment which had been standardized electrometrically. No pH change occurred except when mold growth took place, and in a positive plate the pH was no longer of any importance.

Care was taken to have vigorous strains of the common molds, such as *Aspergillus niger, Aspergillus glaucus, Rhizopus nigricans, Penicillium frequentans,* etc. These organisms were obtained from moldy food products and were grown on raisins moistened with nutrient agar. It was found that raisins furnished an excellent natural medium for cultivating molds. In most cases all the different types were grown on the same plate, and the spores were thoroughly mixed before being used for seeding. Mixed cultures of molds were used to insure that each point determined represented the effect on the most resistant type of common mold.

In the determination of critical points it was convenient to make an orientation series of plates from which was obtained the approximate critical point. The number of plates necessary to obtain the desired point was thereby made much smaller.

DISCUSSION

This study was confined to determination of the concentration of each acid, at various pH values, necessary to inhibit mold growth under one set of conditions of time and temperature of incubation. A change in the conditions, for example, a longer incubation period, would give a slightly different set of results without materially affecting the quantitative relationships of the acids. The vigorous growth in 48 hours on the control plates as compared with no visible growth on the plates containing the critical concentrations of fungistat indicated the marked degree of inhibition obtained.

The fungistatic properties of the saturated acids containing one to nine carbon atoms show that formic and acetic acids have curves lying rather close together (Fig. 1). At pH 2 and pH 3 formic is more fungistatic than acetic. This effect may possibly be due to the reducing power of the formic acid molecule. Above pH 3, acetic is slightly stronger than formic. Propionic, butyric, valeric, and caproic acids form a family of related curves. Enanthic, caprylic, and pelargonic form the third family of curves on this graph. At pH 2 and pH 5 these last three acids have the same points on the graph.

The curves of capric, hendecanoic, lauric, tridecanoic, and myristic acids (Fig. 2) show that capric acid is the first member of the series that does not have fungistatic properties at pH 2. At pH 2,

caprie acid is practically insoluble, and this insolubility may be responsible for its lack of fungistatic properties at this pH.

The fungistatic properties of three acids containing four carbon atoms—butyric, iso-butyric, and crotonic—indicate (Fig. 3) that the branched chain in the molecule lowers the fungistatic properties. The double bond in crotonic acid increases the fungistatic properties slightly as compared with n-butyric acid.

The effect of isomerism on the fungistatic properties of valeric acid (Fig. 4) indicates that the more side-chains, the less effective the acid. A side-chain near the carboxyl decreases the fungistatic properties more than the same side-chain farther away from the carboxyl.

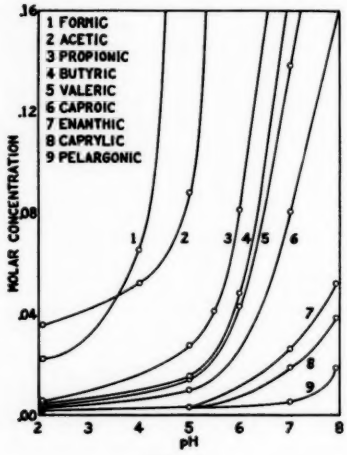

FIG. 1

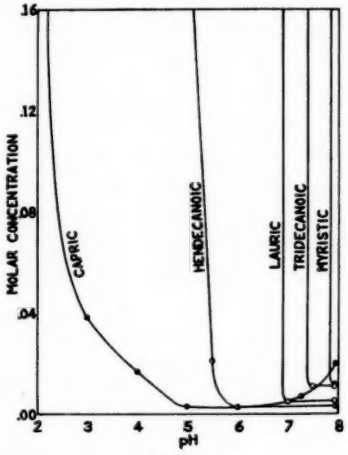

FIG. 2

The relationship between the hendecanoic and hendecenoic acids (Fig. 5) shows that unsaturation in this case increases the effective range of the acid.

The effect of the introduction of iodine in the molecule (Fig. 6) indicates that β-iodopropionic acid is probably both a fungicide and a fungistat. Propionic acid is mainly a fungistat in that it tends to prevent the growth of mold rather than kill it.

POSSIBLE BIOCHEMICAL SIGNIFICANCE

The mold organism, because it is able to grow with vigor over a very wide pH range, at least from pH 2 to 8, is valuable as a test organism in studying the biochemical relationships of the fatty acids. The growth of mold may be considered to be analogous in many

respects to the growth of many other types of cells. A study (Figs. 1 and 2) indicates that at the pH of the blood, about 7.25, the most effective fungistatic acids are those that contain from seven to 12 carbon atoms. These fatty acids which occur naturally in glycerides and essential oils may have important physiological properties because of their power to retard the growth of such an extremely resistant organism as mold.

Peck and Rosenfeld (1938) have reported on the use of the fatty acids as therapeutic agents for fungous diseases of the skin.

The tuberculosis organism contains considerable quantities of fat and this analogy to mold suggested that the effect of the fatty acids on this organism be studied. Preliminary results (private communi-

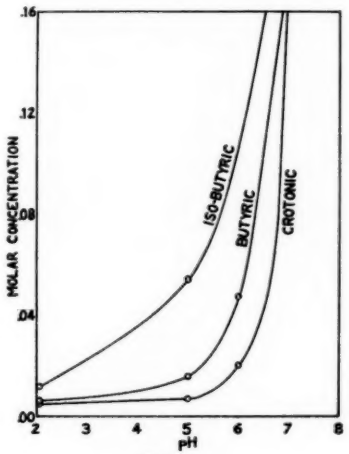

FIG. 3

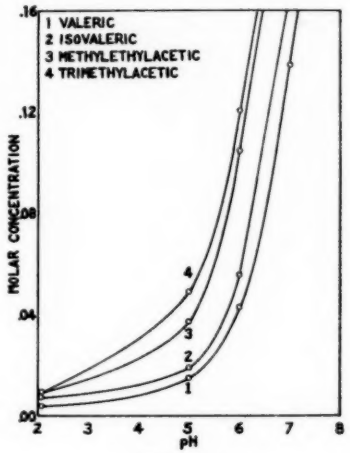

FIG. 4

cation) indicate a degree of inhibition similar to the inhibition of mold. Hailer (1938) reports that *B. tuberculosis* is especially sensitive to caproic, caprylic, enanthic, capric, and pelargonic acids.

The hypothesis is advanced that the fatty acids containing seven to 12 carbon atoms may have some effect on malignancy since this condition is considered by most authorities to be a result of uncontrolled cell division. Helmer and Clowes (1937) have shown that oleic acid has much greater inhibiting action on the active agent of chicken sarcoma than does stearic acid. Their experiments were carried out in a solution of disodium phosphate which would indicate a pH slightly above 8. At such a pH oleic acid shows slight fungistatic properties, while stearic acid shows none. Strong (1938) has shown that heptylic aldehyde has definite anti-carcinogenic prop-

erties. Heptylic aldehyde is closely related chemically to heptylic (enanthic) acid. Enanthic acid, at pH 7.25, retards mold growth but is not as effective as the acids containing eight to 12 carbon atoms. The fungistatic properties of a fatty acid may be indicative of anti-carcinogenic properties.

A very large percentage of the fatty acids present in the glycerides which make up the normal human fat intake are those acids containing 16 to 18 carbon atoms. Even in butterfat about 75 per cent of the fatty acids in the glycerides contain 16 or more carbon atoms. Goat milk fat is an interesting substance because it contains more of the shorter chain fatty acids, in the eight to 12 carbon atom range, than does butterfat. Few instances of tuberculosis in goats

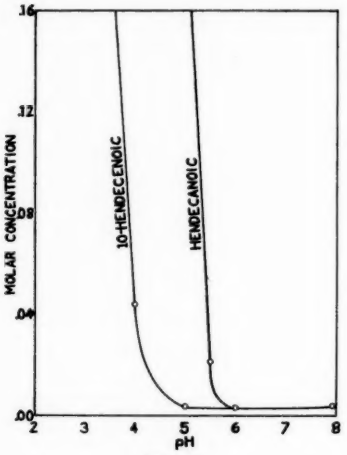

FIG. 5

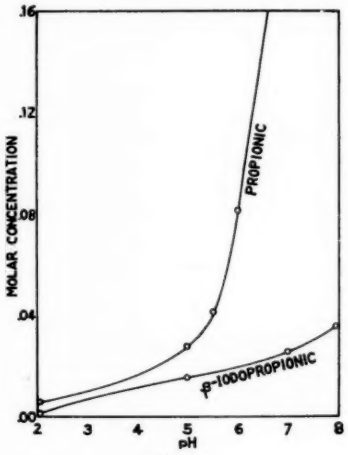

FIG. 6

have ever been reported, and the feeling exists, even among physicians, that goat milk is valuable in cases of tuberculosis. If experimental evidence supports the hypothesis that the acids containing eight to 12 carbon atoms are valuable nutritionally, renewed consideration must be given to the type of fats which enter into our normal dietary.

Work has been undertaken at the Philadelphia Institute for Medical Research and at the Rush Hospital, Philadelphia, on the physiological effect of the shorter chain fatty acids and the corresponding glycerides. For the preliminary experiments capric acid is being given special consideration as it is effective fungistatically over a very wide pH range.

SUMMARY

The fungistatic properties of the normal saturated fatty acids containing from one to 14 carbon atoms have been studied over a pH range of from pH 2 to pH 8. Many of these acids exhibit remarkable effectiveness in the inhibition of mold growth. This effectiveness varies according to the chain length, the concentration of the acid, and the pH of the media. A branched chain acid, in general, is less effective fungistatically than the corresponding straight chain acid. Unsaturation tends to increase the fungistatic effectiveness of an acid.

At neutrality, the acids containing eight to 12 carbon atoms are the most effective for the inhibition of mold growth. This biochemical fact suggested that the nutritional value of these fatty acids be studied in relationship to certain types of disease. The hypothesis has been advanced that the shorter chain fatty acids may be important in the human dietary.

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PROCESSING AND THIAMIN[1,2]

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In an earlier paper Arnold and Elvehjem (1938a) demonstrated that vacuum-packed animal tissues lost relatively little thiamin (vitamin B_1) during a two-year storage period. The antineuritic activity of the tissues was determined by the chick-assay procedure, Arnold and Elvehjem (1938b). More recently a number of investigators—Daniel and Munsell (1937), Fellers (1936), and Fixsen (1938-39)—have been interested in the stability of this and other vitamins to the various steps that enter into the preparation of foods for human consumption. It has previously been observed by the Medical Research Council (1932) that the thiamin content of foods is decreased during canning. Fixsen (1938-39) has noted, however, that studies on thiamin in meats are not extensive. In this connection, therefore, the results obtained in a study on the stability of the antineuritic vitamin under several conditions of processing are of interest. The thiamin content of the meats and meat products used in this study was determined by the chick-assay procedure, Arnold and Elvehjem (1938b), which has facilitated this and other studies by Arnold and Elvehjem (1938a).

EXPERIMENTAL PROCEDURE

Day-old White Leghorn chicks obtained from the University Poultry Department were used in these studies. The method of handling the chicks and the basal ration (Ration 242A) have been described previously by Arnold and Elvehjem (1938b).

The samples[4] may be separated into three groups on the basis of the treatment accorded them. In each case a sufficiently large sample for biological assay was vacuum dried without any previous heat treatment. The control samples were obtained in vacuum-packed two-pound cans. The processed samples were subjected to the following conditions:

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[1] Published with the approval of the director of the Wisconsin Agricultural Experiment Station.

[2] Presented in part at the twenty-ninth annual meeting of the American Society of Biological Chemists, April, 1935. (See reference by C. A. Elvehjem, W. C. Sherman, and A. Arnold. 1935.)

[3] Wilson and Company Fellow.

[4] The animal tissues were supplied by Mr. L. M. Tolman, Wilson and Company, Chicago.

1. The beef spleen, beef-lung tissue, and beef kidney were processed for two hours at 115.6°C.(240°F.).

2. The hog muscle was processed for one hour and 50 minutes at 121°C.(250°F.).

3. Sample A515, a meat food product which contained a small amount of grains, was separated into four additional portions which were treated in the following manner:

a. Processed for one hour at 240°F.
b. Processed for one and one-half hours at 240°F.
c. Processed for two hours at 240°F.
d. Processed for one hour and 50 minutes at 250°F.

In all cases the heat-treated samples were processed in one-pound tins. It should be emphasized that the process used in treatment (a) that is, one hour at 240°F., is lower than is commercially feasible. The samples had a moisture content of approximately 70 per cent and were at pH 6 to 7. The processed samples, together with any accompanying water, were prepared for assay by drying in thin layers on galvanized iron pans in a drying room at 50 to 60°C.(122 to 140°F.) before fans over night. The loss of thiamin under these conditions was negligible. The products were finely ground before use and usually mixed with a weighed portion of the autoclaved grain component of the basal ration.

METHOD OF ASSAY

The dried control and processed samples were assayed for their thiamin content by substitution into the ration at several levels. The lowest level of material necessary to protect all the chicks in the group from polyneuritis was used as the basis for the calculation of the antineuritic activity. It has been previously demonstrated by Arnold and Elvehjem (1938b) that chicks fed Ration 242A require approximately 60 micrograms per cent thiamin in the ration in order to be protected from polyneuritis. A determination of the least amount of the tissue required in the ration to prevent the onset of the polyneuritic syndrome may thus be used for an evaluation of the thiamin content.

RESULTS

Beef-Lung Tissue: Good growth and protection from polyneuritis resulted when the chicks were fed Ration 242A supplemented with 10 per cent vacuum-dried lung tissue (Group 69, Fig. 1). Two of four chicks fed the basal ration supplemented with 40 per cent processed lung tissue (Group 125) were not protected from a thiamin deficiency.

Beef Kidney: The chicks fed Ration 242A supplemented with four per cent vacuum-dried beef kidney (Group 88, Fig. 1) were protected from polyneuritis. One chick of three which were fed the basal ration supplemented with 16 per cent processed beef kidney (Group 120) was not protected from a thiamin deficiency. Good growth and protection from polyneuritis resulted when the chicks were fed the basal ration supplemented with 20 per cent processed beef kidney (Group 121).

Beef Spleen: The chicks fed Ration 242A supplemented with 10 per cent vacuum-dried beef spleen (Group 91, Fig. 2) were protected from polyneuritis. A supplement of 35 per cent processed beef spleen to the basal ration (Group 98) protected three chicks from polyneuritis while a supplement of 25 per cent processed beef spleen (Group 99) proved to be insufficient.

Hog Muscle: The control sample of hog muscle was effective in preventing the onset of the polyneuritic syndrome in chicks at a three-per cent level with Ration 242A (Group 12, Fig. 2). One of four chicks fed the basal ration supplemented with 15 per cent processed hog muscle (Group 11) was not protected from polyneuritis.

Sample A515: Two of four chicks fed the vacuum-dried control sample at a 10-per cent level with Ration 242A (Group 63, Fig. 3) died after exhibiting the typical polyneuritic syndrome. An increase in the supplement to 12 per cent (Group 81) served to protect the chicks during the five-week experimental period.

The chicks fed the basal ration supplemented with 30 per cent of the sample processed for one hour at 115.6°C.(240°F.) (Group 56, Fig. 3) were protected from polyneuritis. A supplement of this sample fed at a 25-per cent level (Group 42) did not protect two of four chicks.

The chicks fed Ration 242A supplemented with 25 per cent of the sample processed for one and one-half hours at the same temperature (Group 43, Fig. 3) were not protected from a thiamin deficiency. An increase in the supplement to 35 per cent (Group 57) protected the chicks from polyneuritis. Lack of material prevented further assays. It may be seen that the low rate of growth of the group indicated a border-line level of thiamin in the diet.

Ration 242A supplemented with 25 per cent of the sample processed for two hours (Group 44, Fig. 3) did not supply sufficient thiamin to protect the chicks from polyneuritis. The chicks fed the basal ration supplemented with 40 per cent of the same sample (Group 59) were protected from polyneuritis. Lack of material prevented further assays. Since the growth rates of the chicks

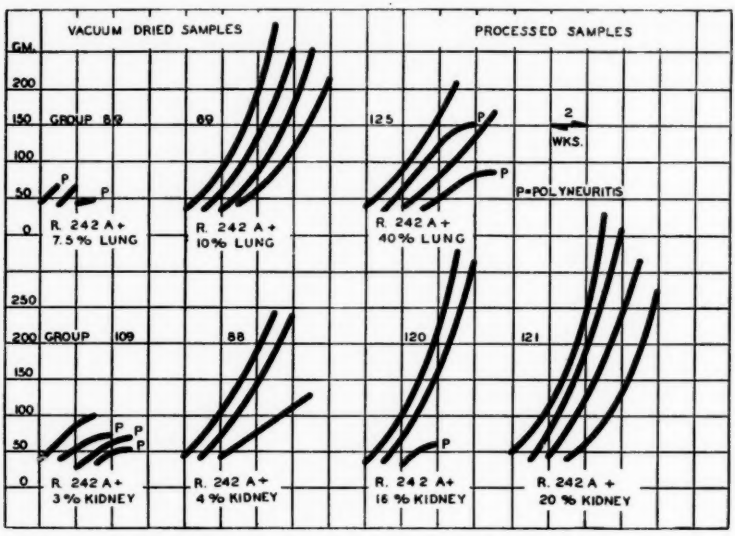

FIG. 1. Individual growth records of chicks fed Ration 242A supplemented with vacuum-dried and processed (two hours at 240°F.) samples of beef-lung tissue and beef kidney.

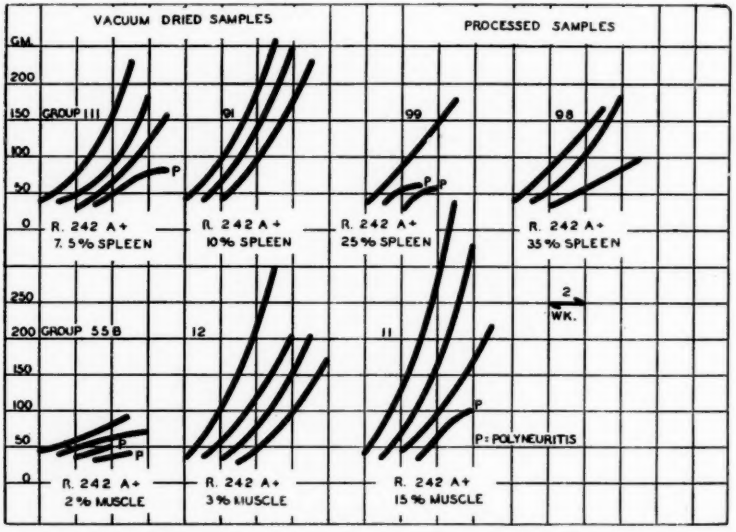

FIG. 2. Individual growth records of chicks fed Ration 242A supplemented with vacuum-dried beef spleen and hog muscle and with processed (two hours at 240°F.) beef spleen and processed (one hour, 50 minutes at 250°F.) hog muscle.

indicated border-line to near border-line levels of thiamin in the ration, calculations were based on the protective level of the supplement.

Two of four chicks fed Ration 242A supplemented with 60 per cent of the sample (Group 65, Fig. 3) processed for one hour and 50 minutes at 121°C.(250°F.) died after exhibiting the polyneuritic syndrome. Higher levels of this supplement were not fed.

Since chicks require approximately 60 micrograms (20 International units) of thiamin chloride per 100 grams of Ration 242A for protection from polyneuritis, Arnold and Elvehjem (1938b), it

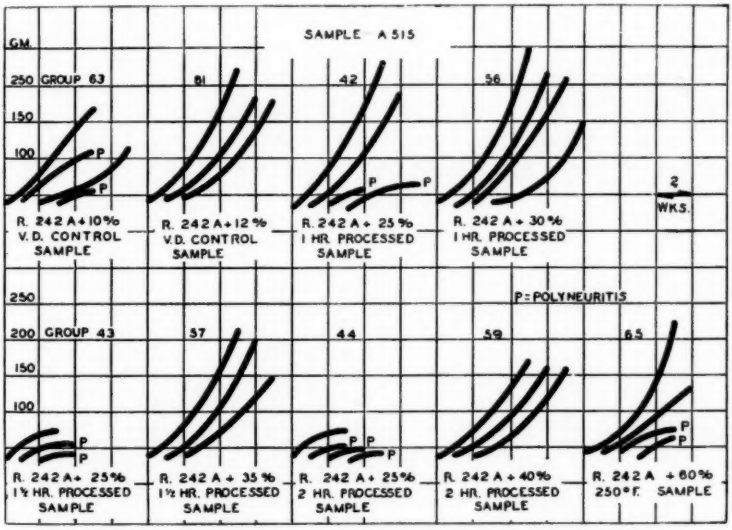

FIG. 3. Individual growth records of chicks fed Ration 242A supplemented with vacuum-dried and processed Sample A515 (a meat product which contained some grains).

is possible to evaluate the thiamin content of the animal tissues both before and after processing and thus to determine the losses during processing (Table 1).

DISCUSSION

The results presented in this communication demonstrate that under certain conditions of processing the antineuritic content of animal tissues may be materially affected. During the processing of kidney, lung tissue, and spleen at 115.6°C.(240°F.) for a two-hour period the decrease in thiamin may be of the order of 70 to 80 per cent. A slightly greater loss resulted during the processing of

hog muscle for one hour and 50 minutes at 121°C.(250°F.). The assays indicated a decrease in thiamin content of over 80 per cent.

The results obtained in the assays on the control and canned samples of A515 indicated that slightly over half of the antineuritic activity had been lost during the first hour at 240°F. An increase in the processing time to two hours only slightly increased the loss in thiamin potency since the sample still contained 1.5 micrograms of thiamin per gram. This was in agreement with the results obtained during the processing of the beef spleen. The temperature

TABLE 1

Stability of Thiamin During Processing of Animal Tissues

Tissue	Treatment	Protective level	Thiamin content per gram dry basis		Decrease in thiamin content
		pct.	*I.U.*	*γ*	*pct.*
Beef kidney	Vacuum dried	4	5.0	15.0	....
	Proc. 2 hr. at 115.6°C.(240°F.)	20	1.0	3.0	80
Beef-lung tissue	Vacuum dried	10	2.0	6.0	....
	Proc. 2 hr. at 240°F	>40	<0.5	<1.5	>75
Beef spleen	Vacuum dried	10	2.0	6.0	....
	Proc. 2 hr. at 240°F.	35	0.6	1.7	70
Hog muscle	Vacuum dried	3	7.0	20.0	....
	Proc. 1 hr., 50 min. at 121°C.(250°F.)	>15	<1.3	<4.0	>80
Sample A515	Vacuum dried	12	1.7	5.0	....
	Proc. 1 hr. at 240°F	30	0.7	2.0	60
	Proc. 1½ hr. at 240° F.	35	0.6	1.7	67
	Proc. 2 hr. at 240°F	40	0.5	1.5	70
	Proc. 1 hr., 50 min. at 250°F	>60	<0.3	<1.0	>80

of processing during the longer periods markedly affected the thiamin content of Sample A515 since the sample treated for one hour and 50 minutes at 250°F. retained less than a fifth of the original vitamin activity. This agrees with the tests on the control and processed hog-muscle samples.

The results demonstrate that thiamin is somewhat more labile to an increase in temperature than to an increase in time. It has been noted by the Medical Research Council (1932) that thiamin is comparatively stable at 100°C.(212°F.) in an acid or slightly acid medium, while considerable destruction occurs in an alkaline medium even at low temperatures. There are other factors possibly affected by processing which cannot be considered here since they are beyond the scope of this communication.

Since animal tissues are excellent sources of the antineuritic factor, according to Mickelsen, Waisman, and Elvehjem (1939), they may remain good sources of the vitamin under carefully operated processing conditions. It is doubtful, however, if the methods can be modified sufficiently to prevent all loss of this vitamin and still bring about complete preservation of the product.

SUMMARY

The thiamin content of beef kidney, beef-lung tissue, and beef spleen was decreased by 70 to 80 per cent during processing for two hours at 115.6°C.(240°F.).

Sample A515, which was a meat food product containing a small amount of grains, processed at 240°F. for periods varying from one to two hours lost increasing amounts of thiamin which ranged from 60 to 70 per cent. The results indicated that the greater portion of the vitamin was lost during the first hour.

Hog muscle and Sample A515 lost over 80 per cent thiamin during one hour and 50 minutes of processing at 121°C.(250°F.).

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HONEY AND THE LEAD CONTAMINATION PROBLEM[1]

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Although lead is apparently not a normal constituent of foods, numerous investigators have reported its presence here at times in significant amounts. Myers, Thorne, Gustafson, and Kingsbury (1933), on surveying the raw and canned fruit and vegetable supply of New York City from the standpoint of contamination by spray residues, found lead and arsenic present. Even some samples of milk which they had examined were not found to be free of the former. Manceau, Griffon, and Breton (1936) detected measurable amounts of this metal in commercial samples of canned beans, peas, tomato juice, sardines in oil, pâté de foie gras, and mackerel fillets in wine and in unsweetened condensed milk. Bishop (1929) claimed to have found it in the eggs of the domestic hen, but Taylor (1930) doubted the validity of his conclusions. Fox (1922) reported large amounts of lead in the calcium acid phosphate used in self-rising flours, and Williams (1927) found it in tinned beef. The Jacksons (1932) described a case of lead-poisoning due to cider; Wirthle and Amberger (1922) observed that tea brewed from leaves removed from a faulty lead-lined package may contain from one to 3.4 mg. of this metal per cup; Kielhofer (1929) found it in the must and the wine from sprayed grapes; and Petherick (1928) called attention to its presence in carbonated beverages.

Seizures of apples, pears, cherries, currants, plums, quinces, cabbage, cauliflower, celery, broccoli, and peppers of high lead content have been frequently made in recent years by the U. S. Food and Drug Administration. A number of fruit products, such as apple butter, apple chops, vinegar, and jam, have also received an official censure as have sardines, canned tuna, anchovies, gelatin, tea, and maple products. Traces of the metal have also been reported in candy, chocolate, cocoa, and chewing gum.

Because of the widespread occurrence of lead in foods and the fact that a literature search yielded no information on the subject with respect to honey, the question arises whether this element might not at times be met with in the routine examination of the latter. If

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this be, indeed, established then its presence can be traced to at least two sources: spray residues which have been worked into the honey by the bees and metallic contamination by processing equipment and improperly soldered containers.

Contamination with lead from insecticides seems probable because of the common practice of spraying fruit trees during the blossoming period with lead arsenate. Then, too, the practice of dusting orchards and vegetable plots with dry insecticides with the aid of airplanes is becoming more and more common. This mode of application offers the possibility of nectar-contamination during much of

TABLE 1

Lead Content of Honeys Sold in the Comb

Predominant floral source	Origin	Crop	Lead content	
			mg. per kg.	*gr. per lb.*
Alfalfa	Wyoming	1930	0	0
Alfalfa-clover	Colorado	1930	.03	0
Alfalfa-clover	Colorado	1930	0	0
Buckwheat	New York	1937	0	0
Buckwheat	New York	1937	0	0
Buckwheat	Wisconsin	1938	0	0
Clover	Ohio	1938	0	0
Clover	West Virginia	1938	0	0
Clover	Illinois	1938	0	0
Cotton	Georgia	1938	.05	0
Gallberry	Georgia	1938	0	0
Mexican clover	Georgia	1938	0	0
Pepper bush	Georgia	1938	0	0
Sweet clover	Wisconsin	1938	0	0
White clover	Wisconsin	1938	0	0
Wonder honey plant	Georgia	1938	0	0

the honey-producing season since there is always the possibility that the powder will drift for considerable distances, thus covering not only fruit trees and vegetable plots but also nearby fields of alfalfa, clover, etc., as well as uncultivated areas where wild flowers are growing.

That bee mortality invariably follows the application of insecticides is reflected in the fact that in some localities the practice is regulated by official mandate in an effort to protect the apiarist against heavy losses. No information as to the toxic effect of lead upon bees is available nor do the reports of McIndoo and Demuth (1926) and Geinitz (1934), that touch upon the lethal doses of arsenic for this insect which apparently lie between .004 and .001 mg., indicate the role played by the former as an insecticide in this

case. Nevertheless, it may be inferred that it is contributive to the gross effect of the arsenate, for Eckert and Allinger (1935) have found that bee mortality is apparently lower when the corresponding calcium compound is used.

The question whether the bee carries insecticides into the hive has not been convincingly answered. Since a bee's power of flight is lost soon after ingestion of arsenic, Eckert and Allinger (1935), nectar gatherers usually die afield. Little or no poisoned nectar, therefore, is deposited in the combs, and honey extracted from brood combs of colonies with heavy mortalities has not been found to kill bees when subsequently fed to them as shown by Eckert (1935). Pollen gatherers, on the other hand, have been observed to carry contaminated pollen to the hive with resultant poisoning of nurse bees and subsequent starvation of larvae. Eagland (1936) found 1.2 parts per million of As_2O_3 in a sample of honey taken from a hive which had suffered heavy mortality following the use of a lead-arsenate spray in a nearby orchard. McIndoo and Demuth (1926) reported somewhat similar results except that only one of 12 samples of honey, produced from nectar gathered from trees which had been sprayed when in full bloom, were found to contain arsenic. The positive indications of arsenic in the cases cited above makes lead a likely concomitant. Quantitative data are lacking, however.

Contamination of foods with lead from processing equipment or containers is not unusual. Examples of container-contaminated foods have been cited above. A food hitherto unsuspected of having been contaminated in processing is maple syrup and its products. In this instance lead-painted sap-gathering buckets and evaporators whose seams and holes had been covered with solder containing lead were found to have been the causative agents.

Honey generally undergoes little processing before reaching the consumer. The practice of removing colloidal matter for the purpose of improving its appearance seems not yet to be general. It is marketed in the original comb without any alteration or as a syrup packed in glass or tinned container which varies in size from the quart pail to the five-gallon can, or "sixty," of the wholesale trade. In the latter case, the honey is removed from the frame of uncapped cells either by straining it through sieves of cloth or metal or by expelling it with the aid of a simple centrifuge or "extractor" housed in a galvanized iron or tinned steel drum. Whatever processing honey may then be subjected to, except as noted above, usually takes the form of heating it to prevent fermentation or retard crystallization, or blending two or more kinds to get a product of uniform color and flavor.

It is conceivable that extracted honey, which is acidic in reaction, might at times absorb lead from soldered seams, painted surfaces, improperly cleaned extractors, blending or mixing kettles, and filtering apparatus.

In order to learn to what extent, if at all, lead is a contaminant of honey, 46 samples were secured for analysis from growers and distributors in 15 states and one territory of the United States. Geographically, every section of the United States was represented. The comb-honey group consisted of 16 samples; the extracted, 30. The latter group included four which were, in part at least, apparently blended products; 19 of them had been purchased directly from growers who are using tinned containers in which to ship their

TABLE 2

Lead Content of Extracted Honeys Sold in Glass Containers

Predominant floral source	Origin	Crop	Lead content	
			mg. per kg.	*gr. per lb.*
Alfalfa	Montana	1930	0	0
Algaroba	Hawaii	1937	0	0
Blue curl	California	1938	0	0
Clover	Wisconsin	1938	0.07	0
Clover	California	1938	0	0
Sweet clover	Wisconsin	1938	0	0
Spike weed	California	1938	0	0
Wild flowers	Virginia	1937	0.05	0
Unknown[1]	Chicago	.....	6.80	.047
Unknown[2]	Chicago	.....	0.17	.001
Unknown[2]	Chicago	.....	0	0

[1] Commercial sample purchased from an independent distributor. [2] Commercial sample purchased from a chain store.

products. The remainder were received in glass. The majority of the lot represented the 1938 crop. There were among the group, also, honeys produced during the years 1930 to 1937, inclusive. Floral sources were varied—there were 21 types in the group—with clover, buckwheat, alfalfa, and tupelo predominating. All the commercial color grades from water-white to dark were represented.

EXPERIMENTAL PROCEDURE

The method of analysis used in this study was the tentative one of the Association of Official Agricultural Chemists (1935) modified to fit present needs. Its major steps consist in ashing in a platinum dish and below 500°C.(932°F.), five to 50 grams of the honey; separation of the lead as the dithizone (diphenylthiocarbazone) complex, Fischer (1929, 1933); and determination of the latter with the

aid of the Evelyn photoelectric colorimeter. At various steps in the procedure provision is made for removal of interfering elements. It is also quite essential that every precaution be taken to ensure the use of water, reagents, and glassware which have been rendered as lead-free as it is possible to get them. It is imperative, too, that blank determinations be made on the reagents and that all data be corrected for the small, though measurable, amounts of lead which apparently cannot be removed by the usual purification procedures.

A 620 mμ filter was used in the colorimeter. The latter was cali-

TABLE 3

Lead Content of Extracted Honeys Received in Tinned Containers

Predominant floral source	Origin	Crop	Lead content	
			mg. per kg.	*gr. per lb.*
Alfalfa	California	1938	0.32	.002
Alfalfa	California	1938	0	0
Alfalfa-sweet clover	California	1938	0	0
Buckwheat	Minnesota	1937	0.91	.006
Gallberry	Georgia	1936	0.45	.003
Holly blossom	Arkansas	1938	0	0
Horsemint	Texas	1938	0	0
Mesquite	California	1938	0.04	0
Orange	California	1938	0.02	0
Sage-buckwheat	California	1938	0	0
Tamarisk	California	1938	0	0
Thistle	California	1938	0	0
Titi	Georgia	1936	4.26	.030
Titi	Georgia	1938	1.91	.013
Tupelo	Georgia	1938	0	0
Tupelo	Florida	1938	0.07	0
Tupelo	Florida	1938	2.47	.017
Unknown	Texas	1938	0	0
White sage	California	1938	0	0

brated against chloroform solutions containing .0104 mg. of dithizone per ml. which had been shaken, under controlled conditions similar to actual determinations, with solutions containing varying amounts of lead. Several sets of galvanometer readings, representing lead contents ranging from zero to 75 micrograms of metal in steps of five, were recorded. A curve was then plotted for reference use in the determination of lead content of the honeys under examination.

The average for 15 blank determinations in which all of the steps and conditions of a regular determination were meticulously observed —in three trials lead-free honeys were used—was 18.5 micrograms (.0185 mg.). In ten recovery experiments in which known amounts

of lead, varying from five to 40 micrograms, were added to honey free of this element an average value of 98.9 per cent was obtained.

RESULTS

Although all determinations were made in triplicate, averages only are herein recorded. Results are expressed in terms of grains per pound, which is the unit commonly used by official agencies, and milligrams per kilogram, the latter permitting finer distinctions. Data have been divided into three groups (Tables 1 to 3) as they are pertinent to comb honeys and to the extracted product packaged, respectively, in glass and tin containers.

Approximately 83 per cent of the honeys analyzed were found to be lead-free or substantially so. In this group, too, are the seven listed as doubtful (Table 4), for none of this number contained more

TABLE 4

Summarized Data on Lead Content of Honey

Nature of container	None	Doubtful	Lead content grouped as indicated			
			.015-.5	.5-1	1-3.57	3.57+
			mg. per kg.	*mg. per kg.*	*mg. per kg.*	*mg. per kg.*
Comb	14	2	0	0	0	0
Glass	7	2	1	0	0	1
Tinned	10	3	2	1	2	1
Total	31	7	3	1	2	2

than .07 mg. per kg., a value deemed to lie within the experimental error of the method which is estimated to be *ca* $\pm$ .1 part per million for a 30-gram sample. In the remainder, or those definitely showing the presence of lead, three contained less than .5 part, one carried *ca* one part, two *ca* two parts, one *ca* 2.5 parts, one over four parts, and one almost seven parts per million. It is significant that the last two contained quantities of this metal larger than the present federal tolerance of .025 grain per pound (3.57 mg. per kg.). If the tolerance were still at .018 grain (2.56 mg. per kg.) a third sample would have become a border-line case.

Although the comb-honey samples were found to be lead-free, this was true of only 82 per cent of the extracted honeys packaged in glass. Curiously enough, that honey which was found to contain almost twice the permissible amount of lead was among the remaining 18 per cent. The honeys sold in tin pails showed a higher percentage of metal contamination than the former group, or 31 per cent. Here too, only one sample contained more than the legal limit of lead.

The results of this survey indicate that lead will be found oftener in extracted than in comb honey, an indication that extracting equipment may be the source of contamination. Metal containers of the type now in use may also be regarded in the same light if lead-containing solders have been used in their fabrication. Another source of lead is apparently that associated with the machinery of the blender or processor, for both of the contaminated samples in the glass-packaged group could be traced to this source.

The question of spray-residue contamination can hardly be considered answered by this study since it is not known with certainty that the honey samples secured for analysis had not been produced from the nectar of sprayed flowers. The probability remains, however, that spray-residue contamination is not a real problem.

The results indicate, also, that honey, if carefully handled and packaged after removal from the comb, need not of itself be regarded as a dangerous source of lead in the diet. Even in the case of the single sample of honey containing 6.8 parts per million of lead it would be necessary to consume 150 grams (*ca* 5 oz.) in order to ingest one mg. of the metal. Because honey is an extremely sweet, concentrated food it is not commonly consumed in large amounts so that the lead intake is not likely to be large. On the other hand, however, the quantity of lead found in several of the samples examined is enough to make significant additions to the lead intake in those cases where the amount ingested because of environmental conditions and other foods is already high.

SUMMARY

If extracting and processing equipment be kept in good repair, there seems to be little likelihood that lead need be considered a contaminant of honey. Contamination from sprays applied to orchards when in bloom and to fields of flowering plants is probably automatically taken care of by natural agencies in that bees soon succumb after having sipped nectar from these sources. Comb honey is uniformly free of lead. When lead is found in extracted honey packaged in glass, its source can probably be traced to agencies beyond the apiary. The same holds true also for the extracted product sold in tin pails and cans, although, of course, in either case responsibility still rests in part with the apiarist who uses old, improperly soldered equipment.

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STUDIES ON BACTERIAL FLORA OF NORTH PACIFIC SALMON[1]

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While a number of investigations have been made on the bacterial flora of marine fish, they have included a variety of species taken from many regions and studied with a considerable difference in techniques. Comparatively few of these studies have been specifically concerned with salmon and still fewer of them with the Pacific species, which differ widely in life cycle and feeding habits from the Atlantic species. Most of these, too, have dealt with relatively small samples and localized sampling.

The value of an extensive investigation covering the principal salmon areas under the direct attention of one worker using one standard set of techniques seems desirable, both from the academic and utilitarian points of view. It seemed desirable, too, in so far as was possible to use techniques previously employed by other investigators in this same field so that fresh data might be more nearly comparable with that already in the literature.

From a utilitarian point of view a study of the bacterial flora of fresh salmon deserves attention inasmuch as the major portion of salmon decomposition with its consequent economic losses originates with the living fish in its native habitat. If the organisms commonly concerned in decomposition are those characteristic of the fish and its environment, study and understanding of such organisms may serve as fixed points from which further investigations may be directed intelligently and profitably. It has been the aim of the present study to inquire specifically into the bacterial flora of salmon. This investigation has been confined to a study of the types found in the surface slime, gills, and anus. It has not been the purpose of this work to deal directly with the decomposition of the salmon. This phase has received considerable attention from other investigators, notably Hunter (1920a), and Fellers (1926). Nor, is it within the scope of this report to develop a complete bibliography of marine bacteriology. That field has been well covered by Bohart (1927), while Griffiths (1937) has more recently reviewed the bacteriology

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of food types of fish. Certain investigations should be cited, however, for comparison and contrast of data or methods.

The most exhaustive studies on Pacific salmon have been those just mentioned of Hunter and of Fellers. From the standpoint of taxonomy, Hunter was interested only in those organisms which produced "foul" odors or indol in fish infusions, discarding all the others as lacking direct significance. However, he does suggest that these discarded organisms may possibly play a role in decomposition by virtue of synergistic relationships. Of 300 original cultures obtained in the Columbia River area all but 43 were discarded. Approximately 50 per cent of those retained as significant belonged to the colon-cloacae group, two per cent were *Bacterium alkaligenes,* seven per cent were *Bacterium formosum,* 14 per cent were pigment producers, and 27 per cent were unidentified. Hunter (1920b, 1922a), later working with cultures isolated from Columbia River and southeastern Alaska salmon, pointed out that the bacteria concerned in spoilage have their natural habitat in sea water, and that the organisms found in the viscera and blood during later decomposition are morphologically similar to those found in the mouth and gills during the first 24 hours. He noted that 72 out of 79 organisms belonged to rod-shaped asporogenous types, and four of the remaining were streptococci.

Fellers (1926), likewise, was interested primarily in studies of organisms concerned in decomposition. He assumed that the fish was fresh up to 24 hours after it had been removed from the water; he made no comprehensive studies on fish prior to that time. Yet, in the same publication he gives figures showing an increase in the bacterial content of salmon slime from an initial count of 370 organisms per milliliter (as indicated by growth on ordinary nutrient agar) to 3,900,000,000 during the first 24-hour storage, and comments that the organisms found in slime are essentially the same as those found in salmon. Furthermore, his observations were confined to fish caught in the Puget Sound area which constitutes a relatively polluted environment as compared with areas adjacent to less thickly populated sections. It is conceivable, then, that his data as well as that obtained by Hunter may not constitute the true bacterial picture of salmon which are taken from areas less subject to pollution.

Duff (1929) states that organisms capable of producing spoilage in salmon are found in the *Bacteriaceae, Bacillaceae,* and *Coccaceae* families, the majority belonging to the first named family. Gee (1927) attributed the spoilage of haddock largely to an aerobic spore-former which he consistently isolated from fresh and spoiled fish.

In considering the surface bacteriology of fish, there is rather widespread belief that the slime normally present on fish surfaces is an important factor in determining post-mortem bacterial changes. Being highly albuminous, it constitutes an admirable medium capable of supporting bacterial growth, and because of its mucoid quality it collects microörganisms freely from the environment.

Gee (1927) found that about 50 per cent of the organisms in live haddock slime fall in the *Bacillus mesentericus* and *vulgatus* groups, with *Proteus, Pseudomonas*, and *Diplococcus* the most numerous of the remainder.

Reed and Spence (1929), who also investigated haddock slimes, report that the genera *Bacillus, Proteus, Pseudomonas*, and *Achromobacter* composed about 75 per cent of the types found. Stewart (1932) obtained 247 cultures from 22 haddock, 140 of which she placed tentatively in *Achromobacter*. She likewise obtained 40 cultures of *Micrococcus* and a number of representatives of *Pseudomonas* and *Flavobacterium*.

Gibbons (1934a) reported *Micrococcus* and *Achromobacter* as the predominant types isolated from fish taken off the coast of Halifax. Bedford (1933a) grouped 70 organisms from North Pacific waters under *Rhodococcus, Micrococcus, Serratia, Flavobacterium*, and *Achromobacter*. Harrison (1929) attributed the discoloration of halibut to *Pseudomonas fluorescens*, also consistently found *Achromobacter, Flavobacterium, Sarcina*, and *Rhodococcus* on the surface of both living and dead halibut. Bedford (1933b) reported that marine chromogenic bacteria, as well as *Pseudomonas fluorescens*, were responsible for the discoloration of halibut.

Sanborn (1930), studying the marine flora of both fresh and frozen fish, stated that the predominant types consistently present on fresh fish were *Micrococcus, Flavobacterium*, and *Achromobacter*. In a later work Sanborn (1932) again stressed the significance of these predominating types, and he further reported that a definite bacterial flora occurs on the surface of fish and that a close relationship exists between Atlantic and Pacific isolations.

The bacteriology of fresh fish tissue aseptically removed was not investigated since various authors are in rather close agreement that living tissue does not harbor viable bacteria. Obst (1919) reported this to be true, findings which were subsequently confirmed by other investigators, notably Gibbons and Reed (1930).

No attempts were made to study the bacterial flora of the intestinal tract since it has been shown by Blake (1935) that this is a reflection of feeding habits and environment. Furthermore, Hunter (1922b) has shown that the intestinal tracts of Pacific salmon on their spawning migration are usually sterile.

While it was not the primary purpose of this work to investigate the physiological activities of any particular group, it was considered worth while, incidentally, to determine certain of the metabolic products of the more frequently encountered organisms—metabolic products that might on further study be utilized as decomposition indices to establish the condition of the fish at the time it was processed.

A number of tests have been proposed to evaluate the condition of raw fish, but the processing of salmon results in such radical changes that they are valueless for establishing the quality of the canned product. Organoleptic tests constitute at present the accepted measures used for this purpose.

Three bacterial metabolites that have received mention are indol, hydrogen sulphide, and volatile acids, the last named originating chiefly through changes on fish fats. It was decided to note whether the predominant types or groups of organisms encountered during the course of the survey exhibited any of the potentialities, at least on laboratory media, with sufficient frequency to warrant later attempts to correlate their presence with the actual condition of the fish.

FIELD TECHNIQUES

Securing the material for study constituted the first problem. Most of the other investigators had employed direct-plating methods for the preliminary isolations. Considering the scale on which these studies were to be ultimately developed this procedure did not appear feasible. Laboratory facilities would seldom, if ever, be available in the field. Provisions for the sterilization of equipment and the preparation of media would be totally lacking. Sampling would have to be carried out under difficult and diverse conditions on slippery boat decks, sometimes in rough seas and drenching rains, clinging precariously to fish traps, and under crowded and cramped positions in canneries; so the use of Petri-plate technique did not seem practical. Furthermore, the transportation of hundreds of these plates in a usable condition over a period of many weeks was in itself a problem. As a substitute, preliminary transplant of the mixed cultures on nutrient agar slants was adopted. Certainly the opportunity for the introduction of extraneous bacterial types would be greatly reduced. The very real possibility of overgrowth of some forms by others was recognized. Continuous refrigeration of all samples from the time they were secured until they were studied in the laboratory was practiced to reduce this to the minimum.

Only actual observation could determine whether or not the above methods were feasible. The Columbia River region could be studied with comparative ease and consequently the methods were first ap-

plied to this area. This seemed especially appropriate since Hunter's and Fellers' studies in that region, using the direct-plate method, could be used as a basis for comparison. The details of these investigations of field techniques will be presented in a later paragraph.

During the summer of 1936 the authors visited canneries in the Columbia River region, the principal king salmon area. Samples were taken from live fish, dead fish before and after reaching the cannery, gear and cannery equipment, and packing ice. Two canneries were sampled on the Oregon side and one on the Washington side. Live fish were sampled in mid-river as they came from the nets, and samples were taken at the various collecting stations up the river. No fish more than 12 hours out of the water was sampled. In all 135 mass cultures were obtained for subsequent study.

Sampling consisted in rubbing sterile cotton swabs on applicator sticks over the ventral, dorsal, and lateral surfaces, the gills, and the anus, cultures from these three sources being obtained on alternate fish. In culturing the anus the swab was thrust deep into the aperture and twisted vigorously. In the case of the gills the operculum was lifted with forceps and the swab was rubbed over and through the gill interstices, being carefully withdrawn so as to avoid any external contact. These were rubbed over ordinary nutrient agar slants; the tubes were sealed, stored, and shipped to the laboratory at refrigerator temperatures. Nutrient agar was selected as the isolation medium on a basis of the experience of many of the other previous investigations before cited, as well as our own preliminary studies.

The reasons for employing agar slants for the initial cultivation of mixed bacterial types have been given. To determine the extent to which overgrowth might be expected, 10 cannery fish were sampled in the field using the direct-plate method. Mass or preliminary transplants from the same fish were transferred to agar slants. Both slants and plates were shipped to the laboratory and retained there at refrigerator temperature. Isolations three months later of the different bacterial types found revealed practically identical types with approximately the same frequency of occurrence. The results show the following numbers and types of colonies obtained by the direct-plate method:

Achromobacter	18
Pseudomonas	8
Micrococcus	6
Flavobacterium	4
Kurthia	3
Aerobacter	2
Staphylococcus	2
Proteus	1

Numbers and types of colonies obtained from mixed-slant cultures:

Achromobacter	23
Pseudomonas	5
Micrococcus	5
Flavobacterium	4
Kurthia	1
Aerobacter	1
Staphylococcus	1
Serratia	2

As a further check, experimental mixtures of known organisms were seeded to slants and retained at low temperatures for varying lengths of time. The mixtures were made from the *Achromobacter, Micrococcus, Flavobacterium, Proteus, Kurthia, and Aerobacter* groups. Isolations were carried out after a month's storage time and repeated at monthly intervals over a period of six months. Even those slants which were retained for more than six months yielded all the original bacterial types upon re-isolation. It is reasonable to expect that some groups would grow faster than others at a given temperature, and the extent to which this would occur would be related in part to the differential between their optima. Isolations from 15 of the mixed cultures on slants shipped from the Columbia River area were made immediately on return to the laboratory. About one month had elapsed, however, before these isolations were effected. The possibility that some of the original bacterial types might have died off in this time interval was recognized. Additional cultures obtained in the field and streaked directly to nutrient agar plates and shipped directly to the laboratory yielded similar groups of organisms with very nearly the same frequency of occurrence. Essentially the same data were obtained from these tubes after six months' storage at refrigerator temperature. These results lent assurance that serious changes owing to overgrowth would not occur, and the slant-culture technique was adapted for future investigations.

During the summer of 1937, the Alaskan areas were visited. Salmon from every large area from Ketchikan to the upper end of Cook Inlet at Anchorage were sampled. Thus, a range of some 2,000 miles was included in this investigation, the only really important large American area omitted being Bristol Bay on the Bering Sea. The same general plan was followed as in the Columbia River studies. So far as possible the number of samples taken in any one area was roughly related to the production of that region. A total of 1,634 mass cultures was taken in these areas.

Three changes from the previous year's routine were put into effect during this year's field work. The Columbia River studies had suggested that hydrogen sulphide production and psychrophilic activities might have important bearings on the spoilage phases. Advantage was taken of the facilities offered by the Territorial Health Department at Juneau to make a number of isolations of low-temperature types directly from fresh material. Results justified this effort. A second significant addition which was put into effect was the substitution of agar containing a hydrogen sulphide indicator in place of the ordinary nutrient agar used the year before. Thus, the hydrogen sulphide-producing ability of mixed cultures as obtained directly from the fish was visibly detectable. The third addition involved a serious attempt to isolate spore-forming anaerobes. The reports of other investigators indicate almost complete absences of these forms. Hunter (1920a) found several in decomposing fish flesh, and Fellers (1926) reported anaerobes as comprising two per cent of his total isolations from slime. A recent publication by Shewan (1938) states that no anaerobes were found in haddock slime, but some members of the group *Clostridium* were isolated from the intestines.

If these forms exist on fish or in water, it seems reasonable they would occur, in part at least, in the spore form. The possibility of isolating these spores would be greatly enhanced if other masking forms could be eliminated or reduced in numbers. It seemed that this might be achieved, partially at least, by natural drying. So cotton swabs, after use as above, were placed in sterile envelopes and sealed for further laboratory investigation. One additional procedure was carried out on a small scale. Rough bacterial counts on sea water were obtained in the various areas by using large swabs which would absorb approximately one c.c. of water. These were squeezed and the contents spread directly onto agar in Petri plates and exposed to natural drying. After incubation at room temperature for 48 hours total colony counts were made. The maximum count obtained was 30, and in several instances no colony development occurred.

LABORATORY WORK

Classification of the organisms consisted in reducing isolated pure strains[4] to their generic groupings. Routine nutrient agar served as the isolation medium.

Approximately 20 per cent of the mass cultures from each of the Alaskan areas were chosen at random for detailed investigation.

[4] The term "strain" is used to designate any pure culture obtained by colony isolation.

In making the isolations several large loopfuls of growth were removed from the original mixed mass culture, suspended in saline, and streaked on nutrient agar in Petri dishes. An incubation period of three days was practiced. Observations indicated this to be sufficiently long inasmuch as plates held over seven days showed no important additional development. Incubation of two to three weeks, of course, might be expected to bring forth certain additional types. Fellers and Hunter, however, used a three-day incubation period, and it was desired to follow as nearly as practicable the procedures used by these workers in order that the data obtained might be compared. Following colony development, all macroscopically different colonies were fished to agar slants, incubated, and labeled with an appropriate code number indicating its history. Gram stains were made on all cultures and the morphological data recorded. From three to four colony types were isolated from each plate. Naturally, there were frequent duplications. This might be considered in a way an indication of type frequency or merely a reflection of relative growth rates. The tables that appear later have been made up with this in mind. In all, 1,838 strains from the Columbia River and Alaska areas were isolated and identified. These represented 15 generic groups [5] exclusive of yeasts.

In addition to the morphological characteristics as revealed by the Gram stain, seven types of differential media were considered sufficient for the identification of the isolated strains. The organisms were cultivated routinely in dextrose, lactose, and sucrose fermentation tubes, litmus milk, tryptone broth for production of indol, medium for hydrogen sulphide identification, and gelatin. These cultures were incubated for five days at room temperature. Chromogenesis was observed on slant cultures.

A survey of the temperature preferences of a large number of the isolated strains indicated that 29°C.(84.2°F.) is near the optimum for the vast majority of strains from fish sources. Many of these indeed were killed or completely inhibited by exposure to 37.5°C.(99.5°F.).

Anaerobic cultivations were carried out by the Fildes-MacIntosh technique. Two plans were used in attempting to separate the spores from contaminating extraneous forms. In the first the dried swabs, collected as described above, were seeded to glucose veal broth, heated to 70°C.(158°F.) for 30 minutes, and incubated anaerobically. Tubes showing growth were streaked on glucose brain agar plates and similarly incubated. The second method involved cultivation of

[5] The generic groups were classified in accordance with Bergey's "Manual of Determinative Bacteriology."

material from the swabs in deep brain tubes with 1:10,000 crystal violet. This was carried through eight successive subcultures. Neither method yielded a single definitely obligate anaerobe strain.

One possibly pertinent suggestion was brought out by these experiments. Almost without exception the unheated swabs showed the presence of viable organisms. On the other hand, this was seldom the case with swabs that had been subjected to heat treatment. Apparently, numerous types found on fish are able to survive protracted periods of dessication. In some of these cases as long as eight months had elapsed before attempts at cultivation were made.[6] This would conceivably have a definite bearing on cannery contaminations and sanitation, since it will be recalled that Hunter demonstrated that cannery flora was identical with that found on fish.

The number of low-temperature types obtained in the field in Juneau was increased by further isolations after return to the laboratory. In both instances, mass cultures were streaked on nutrient agar plates and incubated for a period of five days at refrigerator temperature. Isolated colonies which appeared in this time were seeded to agar slants and retained at low temperature. There was a total of 100 strains obtained. Later, their ability to produce indol and hydrogen sulphide and to liquefy gelatin was investigated at different temperatures. Likewise certain of these strains were tested for lipolytic activity.

Kovacs' (1928) technique was employed for indol detection.

As previously indicated, work on the cultures from the Columbia River area had suggested that the distribution of organisms capable of producing hydrogen sulphide might eventually assume importance. Accordingly, a number of experiments were undertaken previous to the Alaska field work in an attempt to perfect a more accurate and sensitive test. It seemed advisable to substitute for the ordinary nutrient agar slants, a medium on which hydrogen-sulphide production by mixed cultures could be detected easily. Hunter (1937) had recently indicated that the use of bismuth citrate as indicator was superior to the usual lead and iron compounds; however, the work at that time was incomplete and still in the experimental stages. Furthermore, his test was adapted to stab technique in a semi-solid medium.

Our work was undertaken with two objectives in mind: to examine the superiority of bismuth citrate as an indicator, and to develop a medium which would be suitable for the type of field work which was to be carried out in Alaska. Also it was of interest to learn what

[6] In a subsequent investigation 245 out of 300 swabs yielded viable non-sporulating strains after 20 months' dessication at room temperature.

proportion of the strains capable of producing sulphide from laboratory media could similarly function in fish tissue.

Raw fish-infusion broth was prepared, sterilized, and tubed in five-c.c. amounts. Three grams of bismuth citrate were dissolved in 2.5 c.c. of concentrated ammonium hydroxide and made up to a volume of 100 c.c. with distilled water. This solution was sterilized separately by filtration and one drop added to each tube of infusion. One hundred and sixteen cultures were chosen at random for the inoculations. Of these, 39, or 33.6 per cent, were definitely positive for hydrogen sulphide in lead acetate-agar medium. The inoculated fish infusion-bismuth tubes were incubated for seven days. Within that time 70 were definitely positive and 12 were questionably so. To each of the questionable tubes .5 milligram of cystine (in sterile solution) was added, and after two additional days of incubation, these also became definitely positive. Thus, as compared with 33.6 per cent in lead acetate medium, the bismuth indicator yielded 70.6 per cent positives—an increase of slightly over 50 per cent. Whole fish tissue, aseptically removed from freshly caught steelhead, macerated, and suspended in saline, when inoculated with the same strains, gave similar results, the only difference being that the number of positives was slightly increased.

Similarly, Martin's broth agar tubes containing bismuth indicator were inoculated with 70 pure strains. Of these 15, or 21.4 per cent, were positive for hydrogen-sulphide production when tested in lead acetate-agar medium. After an incubation period of five days, 50, or 71.4 per cent, had become positive in the Martin's medium. To prove even more conclusively the superiority of the bismuth compound, 85 cultures were chosen which had previously been found negative for hydrogen sulphide in lead acetate medium, as well as for indol production, litmus milk peptonization, and gelatin liquefaction. These were seeded as above to Martin's medium containing the bismuth indicator, and of these relatively inactive strains, 35, or 41.1 per cent, became positive within five days.

Martin's agar medium was too dark for the ready detection of the slight color changes which occasionally occur, so it was diluted one part to two parts of plain agar. This medium tubed as slants with deep butts constituted the great bulk of material used in Alaska, and on it all mass cultures were taken. In use swabs were smeared on the slant and stabbed into the butt.

In the testing for hydrogen sulphide on the cultures purified and identified in the laboratory, however, Hunter and Crecelius' (1938) recently developed technique was used, although modified in certain respects. As previously stated, Hunter had adapted his

test to a semi-solid medium, and the new technique remained essentially the same. No appreciable change was noted upon the addition of a greater percentage of agar, so solid medium was used throughout in the routine testing. In addition to the higher percentage of agar, a solution of cystine was added previous to sterilization, in such amount that a tube of seven c.c. of medium would contain .5 milligram. There was no evidence that sulphur in the form of H_2S was liberated from the cystine on autoclaving, and the additional sulphur in the cystine enhanced the sensitivity of the test.

A number of the purified strains tested produced a brownish darkening on the surface of the bismuth agar but failed to darken along the stab with the possibility that some compound other than bismuth sulphide was responsible for the color. The Almy technique proved this actually to be hydrogen sulphide.

All of the mass cultures obtained in Alaska [7] were routinely tested for hydrogen sulphide and indol production. While the medium employed contained indicator for the direct detection of the former, it was necessary to add sterile tryptone broth to the tubes for the detection of indol. After addition of the broth the tubes were incubated for four days and the Kovacs' test applied.

The lipolytic activity of microörganisms with reference to their ability to produce oxidative rancidity has been reviewed and discussed by Jensen and Grettie (1937). They state that a correlation exists between numbers of bacteria and tendency toward rancidity. However, lipolytic studies were initiated only recently in connection with the proposed testing for and determination of volatile fatty acids as a criterion of spoilage. A number of the isolated purified strains and a few of the mass cultures were tested according to Turner's (1929) method, one modification being the substitution of fish oil in place of the cottonseed oil which he recommends. The organisms were streaked on the oil emulsion plates and incubated for three days. Some of the low-temperature types were similarly studied in an attempt to determine the possibility of changes occurring at refrigerator temperature. The authors are of the opinion that further investigations in this line might be of considerable significance.

On these bases the data presented in the following section were developed.

EXPERIMENTAL RESULTS AND DISCUSSION

The tables that follow are designed to indicate the comparative data revealed from examinations of samples from live fish, cannery

[7] At the time the laboratory work was being carried out on the Columbia River cultures, hydrogen-sulphide determinations were not considered of great significance and only mass-indol productions were tested.

fish, and water from each area. They have been derived from 1,838 strains isolated from fractions of the mass cultures from each area. The generic percentages indicated (Tables 1 to 3) represent the percentages that these genera constitute of the total number of strains isolated. They should not be interpreted as indicating the relative proportions occupied by these groups directly on the fish or in their environment. However, they should demonstrate the generic types common to the fish, to the environment from which they came, and to that into which they are transported and further, any consistency of distribution. The actual percentage of fish sampled carrying those genera which are indicated in the preceding tables to be predominant are shown (Table 4).

The tabulations show that the 1,838 strains from all sources fall into 15 genera, exclusive of yeasts. Six of these—*Achromobacter, Micrococcus, Pseudomonas, Kurthia, Flavobacterium,* and *Proteus*—persist with such a high degree of consistency as to incline one to accept them as constituting at least a significant portion of the normal flora of salmon. Two of the genera, *Achromobacter* and *Pseudomonas,* were observed in significant percentages from every source sampled—water, live fish, and cannery fish. The other genus, *Micrococcus,* is missed from but two sources, and this may easily be a reflection of the small number of samples taken from these sources.

These results may be compared with those of other investigators, particularly Fellers (1926), Hunter (1920a, 1920b, 1922a), and Bedford (1933a).

Fellers' line of investigation did not parallel these in direction or magnitude. He was dealing primarily with studies on decomposition, and his detailed work was largely confined to the Puget Sound district. In studying the flora of waters, however, from Oregon, Washington, and Alaska *Pseudomonas,* or at least fluorescent rods, comprised 23 per cent of the types isolated from water as compared with 25 to 32 per cent in these studies from central and southeastern Alaska, respectively. Other rod forms were not generically placed in Fellers' studies but undoubtedly a number of these must have belonged to *Achromobacter* which in the present studies amount on an average to 59 per cent of all the groups isolated from water. In all of his studies on salmon of varying degrees of freshness from 24 hours to several days, and from other sources, Fellers has derived 16 genera as compared with 15 from the present studies. He reported *Zopfius,*[8] *Chromobacter,* and *Alcaligenes* not identified in the authors' listings, which in turn indicate *Kurthia, Staphylococcus,* and *Lacto-*

[8] Probably Fellers' *Zopfius* was in reality Bergey's *Kurthia,* and the terminology *Zopfius* is merely the newer nomenclature.

TABLE 1

Comparison of Total Isolations From All Sources—Columbia River, Southeastern, and Central Alaska Areas

Source	*Achromobacter*	*Kurthia*	*Flavobacterium*	*Proteus*	*Pseudomonas*	*Micrococcus*	*Sarcina*	*Rhodococcus*	*Lactobacillus*	*Bacillus*	*Serratia*	Yeasts	*Staphylococcus*	*Streptococcus*	*Aerobacter*	*Escherichia*
	pct.	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*
Columbia River	51.8	3.6	9.0	2.1	3.6	18.8	0.5	1.8	0.2	2.6	1.4	....	1.4	1.0	2.0	0.2
Southeastern Alaska	56.1	10.1	4.2	4.3	7.0	10.0	2.3	0.4	3.0	1.0	0.8	0.8	....	....	....	....
Central Alaska	45.2	4.0	1.3	1.0	25.3	13.1	3.2	1.3	0.4	2.7	1.3	1.2	....	....	....	....
All sources	53.7	8.1	4.9	3.4	8.4	12.6	2.0	0.8	2.1	1.5	0.9	0.5	0.3	0.2	0.4	0.1

TABLE 2

Comparison of Trap Fish and Water Flora—Southeastern and Central Alaska Areas

Source	*Achromobacter*	*Kurthia*	*Flavobacterium*	*Proteus*	*Pseudomonas*	*Micrococcus*	*Sarcina*	*Rhodococcus*	*Lactobacillus*	*Bacillus*	*Serratia*	Yeasts	*Staphylococcus*	*Streptococcus*	*Aerobacter*	*Escherichia*
	pct.	pct.	pct.	pct.	pct.	pct.	pct.	pct.	pct.	pct.	pct.	pct.	pct.	pct.	pct.	pct.
Central Alaska—Trap at Hawkin's Cutoff (live fish)	49.0	6.2	....	1.2	30.0	11.2	....	....	1.2	....	1.2	....	....	....	....	....
Southeast Alaska—Traps A, B, C, D (live fish)	59.0	6.9	3.1	1.2	7.0	12.4	....	....	6.2	3.1	....	1.1	....	....	....	....
Central Alaska—Water at Hawkin's Cutoff, Port San Juan, Shepard Pt.	61.0	3.7	....	....	20.6	14.7	....	....	....	....	....	....	....	....	....	....
Southeast Alaska—Water at Taku, Brunogh I., Prince Wales I., Metlakatla	58.0	3.2	....	....	32.2	6.6	....	....	....	....	....	....	....	....	....	....
Columbia River seined fish	55.2	8.0	5.2	2.6	8.0	13.1	....	2.6	....	2.7	....	....	2.6	....	....	....

TABLE 3

Comparison of Cannery Fish Flora—Columbia River, Southeastern, and Central Alaska Areas

Source	*Achromobacter*	*Kurthia*	*Flavobacterium*	*Proteus*	*Pseudomonas*	*Micrococcus*	*Sarcina*	*Rhodococcus*	*Lactobacillus*	*Bacillus*	*Serratia*	Yeasts	*Staphylococcus*	*Streptococcus*	*Aerobacter*	*Escherichia*
	pct.	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*
Central Alaska canneries.....	34.0	3.0	4.3	1.4	24.6	17.4	4.3	1.4	....	5.8	1.4	2.4	....	....	....	....
Juneau [1] storage.....	70.0	7.2	1.0	4.3	7.7	4.8	1.0	....	2.0	2.0	....	....	....	....	....	....
Ketchikan canneries.....	51.4	11.5	5.2	5.1	6.0	11.0	3.1	0.6	2.8	1.2	1.2	0.9	....	....	....	....
Columbia River canneries.....	51.8	3.6	9.0	2.1	3.6	18.6	0.4	1.6	0.2	2.6	1.4	....	1.4	0.3	3.0	0.4

[1] The cultures obtained in Juneau are tabulated separately here although this district is considered to be in the southeastern Alaska area.

bacillus not noted by Fellers. These in both studies occur with such negligible frequency as to be regarded as incidental and insignificant. The most significant difference between this work and that of Fellers is noted in the absence of *Aerobacter* and *Escherichia* from all the sources sampled during this investigation except the Columbia River district and their infrequent occurrence even there. Fellers reported that these types commonly occur on fresh and in decomposing salmon. Gibbons (1934b) states, however, that members of the colon-aerogenes groups are not normal to the intestinal tracts of marine fishes and that they are rarely met except in fish taken near shore or in polluted waters. This is not quoted to invalidate Fellers' data, but as further suggestive that these groups would not be

TABLE 4

Percentage of Fish by Areas Carrying Predominant Genera

Source	*Achromobacter*	*Micrococcus*	*Pseudomonas*	*Kurthia*	*Flavobacterium*	*Proteus*
Columbia River	pct.	pct.	pct.	pct.	pct.	pct.
Live fish	87	33	22	33	22	8
Cannery fish	80	38	8	9	16	12
Southeastern Alaska						
Live fish	81	32	27	4	6	4
Cannery fish	86	29	12	22	15	10
Central Alaska						
Live fish	80	30	37	10	8	3
Cannery fish	56	42	42	12	12	4

the normal expectancy in clean waters. Since Fellers reported an average count of 76 organisms per cubic centimeter of sea water as compared with the maximum rough count of 30 per cubic centimeter observed in this work, it would appear probable that he was dealing with somewhat contaminated waters where one might reasonably expect the colon-aerogenes types. In view of these findings, the writers are not inclined to accept these types as normal to salmon or its unpolluted environment. *Micrococcus,* while found in lower percentages than in Fellers' work, is definitely a salmon type.

Hunter, whose investigations have been the most extensive in certain respects to date, did not concern himself to any great extent with generic classification. He does emphasize the importance of *Pseudomonas* as commonly occurring on fish, in which the authors concur. In addition, he indicates a wide distribution of the colon-aerogenes group with which this work, as indicated above, is in disagreement. Hunter's examination of water samples collected from

six sources in the Pacific Northwest and in southeastern Alaska led him to the belief that the types found on fish are a reflection of environment and that all types in canneries so originate. The writers are inclined to agree with the first part of this reasoning but do not believe that the latter is necessarily true in its entirety. There seems to be no good reason why a variety of types from any of a number of sources may not become established in a cannery.

Reed and Spence (1929) published findings on haddock in considerable agreement with these investigations except that, curiously enough, the relative percentages of *Proteus* and *Achromobacter* are reversed.

The most comparable data are those of Bedford, the one serious discrepancy lying in his refusal to admit *Pseudomonas* to that group of genera of North Pacific types. The writers are somewhat in disagreement in view of isolations of this group from every source from the Columbia River to Cook Inlet.

There seems apparent from a study of the literature no bacterial types specific for salmon. All of the types encountered on salmon and from salmon waters have been reported on other species of fish in other waters. Sometimes one group may be reported as dominant and again some other. In almost all cases, however, small numbers from isolated areas were investigated. It is significant, though, that in practically every instance these fall within the six groups emphasized in this work. In this work, too, some of these variations in generic dominance have been observed from locality to locality or source to source, with the exception of *Achromobacter* which has held an impressively commanding position irrespective of source or area. This places further emphasis on the demand for large-scale sampling.

The magnitude on which these studies have been conducted harmonizes these apparent contradictions. The basic data have been derived from samples of live fish from widely separated sources at the moment of leaving the water. Using this as a base, the generic structure has been constructed from additional observations on water types and cannery flora. The writer feels safe in the assertion that the genera *Achromobacter, Micrococcus, Pseudomonas, Kurthia, Flavobacterium,* and possibly *Proteus* constitute at least a significant portion of the normal flora of salmon and their environment. The frequency with which they will be encountered should be in the order named, although the respective positions of *Pseudomonas* and *Micrococcus* may be reversed as may those of *Flavobacterium* and *Kurthia.*

It follows from this that there should occur no differences in the groups characteristic of the various salmon species, and that reasoning is corroborated by analysis of the data by species. Reference to results (Tables 2 and 3) indicates that any difference existing between seined and trapped fish must be quantitative and not qualitative. It must be realized that the numbers of genera might eventually be increased by new studies on the genus *Achromobacter* which, it must be admitted, constitutes a rather loosely organized taxonomic group possessing under present nomenclature 84 species. It is possible that this group should receive more attention from the salmon industry as being of possible significance in early decomposition. Thirteen of the species of *Achromobacter* listed by Bergey have been isolated from either fish or sea water. Thirty-five per cent of the listed species are gelatin liquefiers; 48 per cent of the strains tested in these studies indicated an ability to attack fish oil; and 64 per cent of the strains listed here produced hydrogen sulphide with 21.5 per cent showing gelatin liquefaction. Any group so dominant numerically should be dismissed only after serious investigation.

The same reasoning applies to the low-temperature types. No attempt has been made in these studies to classify these organisms. Some fall into groups already named and others remain unidentified. The point of interest was the demonstration of their existence on freshly caught fish and their possible ability to attack tissues so far as that ability can be inferred from their proteolytic activities. Whether temperature changes really influence the products formed or merely the rate of formation, the fact remains that tissue invasion may not need to await development of higher temperatures. It potentially begins at the moment of death, proceeding conceivably unnoted at first but preparing the tissues for subsequent broader and more objectionable changes. Particularly might this group be of interest to people in that branch of the industry dealing with frozen fish.

Before considering the possible application of certain metabolites as indices of spoilage, it should again be emphasized that an attempt was made only to determine the distribution of certain groups of organisms capable of producing these metabolic products. No effort was made to correlate organisms of their products of metabolism with any specific physical or chemical state of the fish.

Tabulations of selected biochemical activities of mixed cultures are given (Table 5) in an attempt to show that organisms which may be capable of producing significant metabolic products are widespread and common to all sources. A study and comparison of these

activities indicates the potentialities that certain metabolic products may have as indices of decomposition.

As previously stated, the presence of indol has been suggested by some investigators as an index of spoilage. The results of this study argue against its acceptance. It has been demonstrated (Table 5) that the distribution of indol-producing types is probably less uniform than has hitherto been suspected. Loeffler [9] has demonstrated that indol or substances giving rise to positive indol tests are formed by various cleavages during processing. These facts are sufficient to discourage acceptance of the presence of indol as an adequate criterion of spoilage.

Hydrogen sulphide has been suggested as a second possibility. From the point of view of distribution, the fact that organisms capable of producing it were present on 93 per cent of the nearly 2,000

TABLE 5

Percentages of Fish Carrying H_2S Positive and Indol Positive Strains

Source	Positive for hydrogen sulphide	Positive for indol
	pct.	*pct.*
Central Alaska—live fish	88.8	47.6
Southeastern Alaska—live fish	95.0	34.6
Central Alaska—canneries	89.0	47.6
Southeastern Alaska—canneries	98.0	75.9
Total Alaska	93.8	53.2
Total Columbia River	96.0	64.0

fish sampled over a wide range should render it acceptable from that important initial consideration. That a large percentage of the dominant types occurring naturally on salmon can produce it from fish tissue unaltered by cooking has also been indicated. The ability to produce hydrogen sulphide is also possessed by many organisms which may have strong powers of tissue invasion if the ability to liquefy or to peptonize are any criteria. Furthermore, relatively simple and specific tests are available for its qualitative and quantitative determination.

Within the limits of sensitivity of the tests used in this work there has been encountered no evidence that it is released from fish tissue during processing. On the other hand, there are unknown possibilities. Just what happens to the preformed hydrogen sulphide in the can during processing is a matter of conjecture. It is entirely possible that metallic sulphides may be formed. If so, this

[9] Personal communication, May, 1937.

might or might not present an insuperable objection. A further possible objection might lie in the inability to correlate the amount of hydrogen sulphide with the physical state of the product as indicated by sensory impression. It would seem that sensory tests, then, must continue to occupy a definite part of inspection programs. This is acceptable within certain bounds. In case of gross decomposition such tests are adequate, since it does not appear probable that badly spoiled material will pass the inspector's nose. It is in determining the actuality of finer distinctions that the senses need reënforcement and verification so that the possibility of unjust condemnation of sound material will be lessened. Further, cases of correct judgment would be so substantiated as to remove the elements of suspicion and controversy now existing between the production and inspection branches of the industry.

TABLE 6

Percentage Showing Lipolytic Action at 20°C.(68°F.)

Groups	Lipolytic activity—positive
	pct.
Proteus	100.0
Pseudomonas	74.0
Achromobacter	48.0
Micrococcus	6.2
Flavobacterium	0.0
Kurthia	0.0
Mass cultures	51.8

The possibilities derived from the studies on lipoclastic action might be briefly summarized. The data (Table 6) so far obtained are meager.

As in the case of the other suggested indices a large number of positive results were observed. While the proportion of positives was not so great with this type of action as was noted in the case of hydrogen sulphide, it is of sufficient magnitude to invite further study, particularly when it is called to mind that a relatively small number of strains and mass cultures were studied.

Again the effect of incubation at 6.5°C.(43.7°F.) was noted. Observations were made after six days, at the end of which time 35 per cent of the strains were positive. It is interesting to note the definitely superior ability of the tested strains to bring about this type of change at low temperature as compared with the ability of strains in these same groups to produce hydrogen sulphide at the same temperature. Based upon these observations, lipoclastic action seems to be a property rather widely distributed among the pre-

dominant bacterial groups found on salmon. A variety of products, such as peroxides, free fatty acids, aldehydes, and ketones, may arise as a result of this type of decomposition. Loeffler [10] has shown an increase in acidity coincident with spoilage. This could arise from the action on unsaturated fats. Pertinent to this, Brocklesby (1931, 1933) has shown an increase in the degree of unsaturation in salmon fats the farther north the fish are caught. This might introduce a complicating factor. Utilization of these possibilities is ultimately a chemical rather than a bacteriological problem. The suggestions herein made are sufficiently pertinent, however, to encourage more detailed research on the products of lipoclastic action.

In conclusion, the writers express the conviction that not too much effort be devoted to evolving a single, final and absolute criterion; but rather, inasmuch as spoilage proceeds by various mechanisms, that a group of indices indicative of these several mechanisms be considered.

SUMMARY AND CONCLUSIONS

A total of 1,769 mass cultures was obtained from salmon of North Pacific waters, from which representative proportions 1,838 strains were isolated and placed into 15 genera, exclusive of yeasts: *Achromobacter, Kurthia, Flavobacterium, Proteus, Pseudomonas, Micrococcus, Sarcina, Rhodococcus, Lactobacillus, Bacillus, Serratia, Staphylococcus, Streptococcus, Aerobacter,* and *Escherichia.* In addition, a number of low-temperature types were isolated. No evidence of obligate anaerobes was obtained.

Six of these genera—*Achromobacter, Micrococcus, Pseudomonas, Kurthia, Flavobacterium,* and *Proteus*—persisted from every source and are offered as the normal expectancy. They likewise probably constitute a definite portion of the normal environmental flora. The remaining genera are regarded as more or less incidental. The bacterial groups found on live salmon and on salmon during the first 24 hours out of water are essentially the same as those reported in salmon and undergoing incipient and advanced decomposition with the exception of the colon-aerogenes group.

Within the limitations of the techniques used in this investigation there was no evidence of any bacterial groups peculiar to salmon, and no differences were noted in the bacterial groups carried by the different species of salmon.

A study was made of the abilities of certain pure strains and mixed cultures taken directly from fish to produce certain metabolic products: indol, hydrogen sulphide, and products of lipoclastic

[10] Personal communication, May, 1937.

action. The possible utilization of these products as indices of spoilage has been discussed. Indol has been indicated as inadequate as a criterion of decomposition, primarily because it may arise through cleavages during processing. Further, this study has shown that not only is the number of organisms capable of producing indol limited, but, as indicated by the mass cultures, their distribution is not uniform. Hydrogen sulphide, so far as this study is concerned, offers promise. Its eventual utilization must be governed by other considerations some of which have been suggested. A brief survey of lipoclastic action indicates it as a potentially valuable, though new, field, and an extension of studies in this line is urged.

It is suggested that, as a measure of the kind and extent of decomposition, a group of indices be adopted representing several types of bacterial activity rather than a single index.

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VITAMIN VALUES OF GARDEN-TYPE PEAS PRESERVED BY FROZEN-PACK METHOD. II. VITAMIN A[1,2]

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Green vegetables are recognized as being comparatively rich sources of vitamin A, and with the increasing use of frozen-packed products it becomes important to determine the vitamin content of vegetables preserved by this method.

Little information is available on the vitamin A value of green peas either fresh or preserved. Daniel and Munsell (1937) quote a value of 400-755 Sherman units of vitamin A per 100 grams of green peas. Eddy, Kohman, and Carlson (1926), using ungraded raw green peas from the New York market and canned peas, reported values that are equivalent to 400-2,500 Sherman units per 100 grams of peas. Fitzgerald and Fellers (1938) reported a value of 600-3,300 International units per 100 grams of fresh green peas and 4,800 International units per 100 grams of frozen peas. These values were calculated from carotene determinations and no information was given as to the source or variety of the peas.

There is no generally recognized factor for converting Sherman units to International units nor is the vitamin A value as calculated from the carotene content always the same as that obtained from biological assay, as shown by Taylor and Russell (1938) and Kemmerer and Fraps (1938); therefore, it is impossible to compare these values.

The work here reported is a continuation of studies on the vitamin content of frozen-pack peas by Todhunter and Sparling (1938). In this investigation the biological method was used to determine the vitamin A value of frozen-pack peas of the garden type. The following factors were studied: varietal differences, distribution of the vitamin in the peas, and conditions of handling, such as scalding at different temperatures and for varying periods of time before freezing, freezing in brine solution, and cooking.

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[2] This work is part of a coöperative project with Oregon State College and the next paper (III) in this series will appear shortly, giving the vitamin B_1 content of these same peas.

EXPERIMENTAL PROCEDURE

Materials: The peas were obtained through the courtesy of H. C. Diehl of the Frozen Pack Laboratory, U. S. Bureau of Chemistry and Soils, at Seattle, Washington. Growing conditions and handling methods of all peas were the same as described by Todhunter and Sparling (1938). The peas assayed in Series I, II, and III are also the same peas for which vitamin C values were reported in that paper. The Dwarf Alderman variety assayed in Series IV was produced in 1937, and the Tall Alderman variety in Series V was grown in 1938.

The frozen-pack peas were stored at approximately —15°C.(5°F.) until used in the feeding studies. In the first series the peas were weighed in the frozen state and fed immediately. For the remaining assays the peas were thawed in sealed containers at 40°C.(104°F.) for 45 minutes, drained for one minute on a sieve (United States Standard No. 8), chopped finely on a board, and thoroughly mixed. Since each container held approximately 300 grams of peas, representative sampling was obtained in this way. Individual portions were weighed in small covered dishes and held for not more than five days at —15°C.

For the cooking studies of Series IV the peas were thawed and drained one minute, and a 400-gram sample was introduced into boiling water in a pyrex beaker containing sufficient water to cover the peas, which were then cooked for exactly eight minutes from the time the water returned to boiling. The peas were again drained and weighed. In every case a loss in weight occurred during cooking, and this loss averaged 14 per cent of the weight before cooking, so that the daily feeding of cooked peas equivalent to .15 gram of uncooked peas amounted to .13 gram.

The assay feedings were on the basis of a six-day week (Sunday excluded), but since vitamin A is readily stored in the body and the amounts of the daily feedings were small, the error in weighing and handling was reduced by feeding the pea supplements in double amounts three times per week.

The Tall Alderman variety of peas used in Series V had been frozen by two different procedures—one lot was frozen in the dry state as for all the previous samples, and one lot from the same field plots had been frozen in a 7½° salometer brine solution.

The pea supplements were fed at levels of .1 and .2 gram daily for the first three series and .075 and .15 gram for the remaining series.

Standard of Reference: Every group of test animals receiving pea supplements was matched for sex, weight, and litter mates by

a group receiving a measured amount of United States Pharmacopeia (U.S.P.) reference cod liver oil containing 3,000 U.S.P. XI vitamin A units in one gram of oil. Each animal received daily .1 ml. of diluted oil containing one (or in some cases two) International unit. The diluted oil was freshly prepared each week by weighing .083 gram of reference cod liver oil into a 25-ml. volumetric flask, diluting to volume with Wesson oil at room temperature, 23.89°C.(75°F.), and shaking for 10 minutes to mix thoroughly. This diluted oil was then divided into six vials and stored in a dark container in the refrigerator at 7.22°C.(45°F.). Each day (six days per week) one vial was brought to room temperature, and .1 ml. was fed directly into the mouth of each animal from a glass syringe graduated in 1/100 ml.

Animals: Albino rats which were raised in this laboratory were placed on the vitamin A-free diet, as described by the U. S. Pharmacopeia XI, at 21 to 28 days of age. They weighed 40 to 50 grams and were continued on this basal diet alone until xerophthalmia occurred and weight was constant or declining and then distributed in groups receiving the following supplements: (1) basal diet only, (2) reference oil, (3) frozen-pack peas. The assay groups were matched for sex, weight, and litter, and nine or 10 animals were assigned to each group. Data were calculated for those animals which gained between 12 and 60 grams during the four weeks experimental period, as described in the U. S. Pharmacopeia XI.

Curve of Reference: A curve of reference was established for the laboratory by using 50 rats and feeding to groups of 10 animals exactly matched for weight, litter, and sex such amounts of the standard reference oil to provide the following U.S.P. XI units each day: 1, 1.5, 2, and 3. During a four weeks' assay period these groups gained, respectively, 21, 40, 43.5, and 60 grams.

RESULTS AND CONCLUSIONS

The data from all the groups receiving frozen-pack peas as a supplement to the basal diet and the data from the corresponding control groups receiving standard cod liver oil are summarized (Table 1). In Series I, II, and III the assays were made in the winter and spring of 1937; there was close agreement in growth response from the control groups receiving the reference cod liver oil and fairly comparable growth response from all animals in these series which received .1 gram of frozen-pack peas. By direct comparison with the animals in each group receiving the reference cod liver oil, it was estimated that there were 10 International units per gram of peas. It, therefore, appeared that peas scalded in steam or in water at 99°C.(210.2°F.) for one or for two minutes (Lots

TABLE 1

Vitamin A Value of Frozen-Pack Peas of Several Varieties and Conditions of Handling

Supplement fed	Amount of supplement fed daily	Number of animals	Depletion period	Average weight			Vitamin A value	
				Beginning depletion period	Beginning assay period	Gain in four weeks	Calculated from data within the series	Calculated from reference curve
	gm.		*days*	*gm.*	*gm.*	*gm.*	*I.U. per gm.*	*I.U. per gm.*
Series I								
Negative controls	.....	10	27	44	109	—37		
Cod liver oil	2 (I.U.)	9	27	43	110	40		
Peas—Telephone No. 1724	.1	5	26	43	98	26	10	7
No. 1725	.1	7	27	42	102	30	>10	8
No. 1725	.2	8	27	41	101	53	>10	7
No. 1723	.1	6	26	43	99	23	10	7
Series II								
Negative controls	.....	11	26	46	112	—41		
Cod liver oil	2 (I.U.)	10	26	48	104	38		
Peas—Telephone No. 1730	.1	4	26	45	96	19	<10	6
No. 1734	.1	6	26	49	111	20	<10	6
Series III								
Negative controls	.....	7	26	47	126	—42		
Cod liver oil	1 (I.U.)	4	27	46	119	23		
Cod liver oil	2 (I.U.)	7	26	44	111	35		
Peas—Telephone No. 1727	.1	6	26	49	116	32	>10	9
No. 1729	.1	7	26	47	113	23	10	7
No. 1733, skin	.1	7	26	48	133	25	10	7
No. 1733, cotyledon	.1	6	25	45	122	27	10	7
Series IV								
Cod liver oil	1 (I.U.)	5	38	44	160	15		
Peas—Dwarf Alderman	.075	6	36	47	175	25	14	16
Dwarf Alderman	.15	6	39	44	148	32	14	13
Dwarf Alderman, cooked	.13	7	35	43	141	30	14	13
Series V								
Cod liver oil	1 (I.U.)	7	31	43	108	32		
Peas—Tall Alderman, brine packed	.075	6	32	45	136	29	<13	10
Tall Alderman, dry packed	.075	7	32	43	129	28	<13	10
Tall Alderman, dry packed	.150	10	33	45	128	35		

1723, 1724, 1725) retained the same vitamin A value, and that scalding for two or six minutes at 88°C.(190.4°F.) (Lots 1727 and 1729), or holding for four or eight hours before scalding (Lots 1730 and 1734) caused no further loss in vitamin A value.

There was no difference in weight gain in the groups of animals fed the skin or the cotyledon of one sample of frozen-pack peas of the Telephone variety; each contained approximately 10 International units per gram.

In Series IV the animals in the group receiving supplements of cooked peas made similar gains to those animals receiving equivalent amounts of the raw frozen-pack peas; there was, therefore, no loss in vitamin A value during the cooking process. The Dwarf Alderman variety contained approximately 14 I. U. per gram and, therefore, had a higher vitamin A value than the Telephone variety and was slightly richer in vitamin A than the Tall Alderman peas which contained approximately 13 I. U. per gram.

The Tall Alderman peas, either frozen packed dry or in brine, produced no difference in growth response of the test animals; the groups gained 28 and 29 grams when fed .075 gram daily throughout the four-week experimental period. By direct comparison with the reference cod liver oil groups these peas contained approximately 13 International units per gram.

The last column (Table 1) shows the units of vitamin A obtained when the growth data were interpreted from the reference curve prepared in this laboratory by feeding four different levels of standard cod liver oil, calculations from the reference curve being made according to the method of Coward (1938). Vitamin A values obtained in this way are a little lower than those obtained by direct comparison with control animals receiving the reference cod liver oil.

SUMMARY

The vitamin A value of garden peas preserved by the frozen-pack method has been determined by biological assay according to the United States Pharmacopeia XI method.

No difference was observed in the vitamin A value of Telephone peas which had been scalded at different temperatures and for varying periods of time before freezing; the skin and the cotyledon of these peas contained the same amount of vitamin A per unit of weight. Cooking by boiling caused no change in the vitamin A value of Dwarf Alderman frozen peas.

Some varietal difference was observed, Dwarf Alderman being higher and Tall Alderman slightly higher in vitamin A value than the Telephone variety.

The average vitamin A value for three varieties of peas preserved by the frozen-pack method was 10 International units per gram.

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VITAMIN C CONTENT OF VEGETABLES. XII. BROCCOLI, CAULIFLOWER, ENDIVE, CANTALOUP, PARSNIPS, NEW ZEALAND SPINACH, KOHLRABI, LETTUCE, AND KALE[1]

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A search of the literature reveals little information concerning the vitamin C content of freshly harvested broccoli, cauliflower, endive, cantaloup, parsnips, lettuce, and kale. Still fewer data have been published on the rate of loss of the vitamin from these vegetables under various storage conditions and on the influence of variety and maturity on the vitamin C content.

McHenry and Graham (1935) and Roe (1936) have reported on the vitamin C content of broccoli. Their results were in close agreement, .68 and .68 to .71 mg. per gm., respectively. More recently, Feener, Palmer, and Fitzgerald (1937) made a survey, throughout one year, of broccoli as it arrived in Boston for the market. They also studied the change in ascorbic acid content after storage for 24 and 48 hours at temperatures just above freezing—1 to 3°C.(33.8 to 37.4°F.)—and at average room temperatures—21 to 22°C.(69.8 to 71.6°F.). The values obtained were about twice as high as those of the investigators mentioned above.

The ascorbic acid content of fresh raw cauliflower has been studied by several workers. In India, Ahmad (1935) found only .058 mg. per gm., but Ranganathan (1935) reported over ten times as much. Roe's (1936) results by the indophenol method and by his own furfural method checked very closely (.44 and .45 mg. per gm., respectively), but this value was more than twice that given by McHenry and Graham (1935) for the reduced form of ascorbic acid. Chi and Read (1935) found a vitamin C content for cauliflower of .42 mg. per gm. Somewhat higher values were reported by Fernandez and Alfageme (1936) (.77 mg. per gm.), Zarinn and Putnina (1937) (1.15 mg. per gm.), and Lunde (1938) (.6 mg. per gm.).

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No information was available concerning the vitamin C content of endive with the exception of one investigation by von Hahn (1931), showing 20 Sherman units per 100 grams by biological assay.

TABLE 1

Biological Assay of Ascorbic Acid in Broccoli, Cauliflower, and Endive

(Twelve days' depletion, followed by 21 days' test feeding)

Product fed	Number of animals	Vitamin supplied daily for 21 days	Average weight of depleted animals	Average gain in weight during 21 days	Average scurvy score
		mg.	*gm.*	*gm.*	0-24
Endive, 6.25 gm.	10	.50	367	80	4.3
Cauliflower, .79 gm.	9	.50	368	101	3.3
Broccoli, .78 gm.	9	.50	360	114	3.4
Ascorbic acid, .50 mg.	10	.50	370	86	3.4
Negative controls	4	.00	398	—84	21.5

The ascorbic acid content of cantaloup was reported by Chi and Read (1935) as .015 mg. per gm. in the reduced form, .059 after treatment with hydrogen sulfide, and .14 by the iodine titration method. Ghosh and Guha (1935) found .03 mg. per gm. by the indophenol method and .053 by the iodine titration. Stone (1937) reported .53 mg. ascorbic acid per gram of the fresh vegetable. More

TABLE 2

Biological Assay of Ascorbic Acid in Parsnips

(Twelve days' depletion, followed by 21 days' test feeding)

Vitamin source	Ascorbic acid fed per day	Number of animals	Average weight at beginning of assay	Average weight after 21 days	Average gain in weight	Average scurvy score
	mg.		*gm.*	*gm.*	*gm.*	0-24
Parsnips (2.5 gm.)	0.5	7	291	317	26	5
Ascorbic acid	0.5	8	291	342	51	5
Ascorbic acid	1.0	5	289	382	93	1
Negative controls	0.0	4	303	198[1]	—105	20

[1] Weight at end of survival period.

recently, Floyd and Fraps (1939) reported the ascorbic acid content (in mg. per gm.) of certain varieties of cantaloups as follows: Hale's Best, .33; Hearts of Gold, .24; Honey Ball, .31; Netted Gem, .37; Rocky Ford, .26. They also gave the vitamin C content of the Honey Dew and "big muskmelons" as .07 and .12 mg. per gm., respectively.

McHenry and Graham (1935) found the vitamin C content of parsnips to be .05 mg. per gm. after treatment with hydrogen sulfide, increasing to .061 mg. per gm. on cooking. Tauber and Kleiner (1935) reported .40 mg. per gm. The ascorbic acid content of Norwegian grown parsnips, according to Lunde, (1938) is .20; Mathiesen and Aschehoug (1937) found .16 in the raw and .13 mg. per gm. in the canned vegetable.

Bessey and King (1933) reported .05 mg. per gm. as the vitamin C content of lettuce, and this value was quite closely checked by Chi and Read (1935). Munsell and Kennedy (1935) reported that the outer green leaves of Iceberg lettuce contained slightly less vitamin than the inner bleached leaves. In both cases the daily minimum protective dose for guinea pigs was approximately 21 grams. On

TABLE 3

Ascorbic Acid Content of Freshly Harvested Broccoli

(Expressed in mg. per gm.)

Variety	Whole plant			Blossoms		Stems	
	Number of analyses	July	Sept.	Number of analyses	July	Number of analyses	July
Italian Early Calabrese	11	1.11	1.41	10	1.41	10	1.04
Italian Green Sprouting	3	1.18	1.84	1	1.60	1	1.09
Propagane	6	1.30	1.59	4	1.35	4	1.08
Emerald Isle	9	1.15	1.72	5	1.46	5	1.13
Average or total	29	1.19	1.64	20	1.46	20	1.09

the assumption of a daily requirement for guinea pigs of approximately .50 mg. of vitamin C, an ascorbic acid content of only .03 mg. per gm. was indicated. The value obtained by McHenry and Graham (1935) was only .003 mg. per gm. for the reduced form, and .04 mg. per gm. for the total reduced and reversibly oxidized ascorbic acid. Ahmad (1935) found .005 mg. per gm. Stone (1937) found South African lettuce to be much richer in vitamin C, reporting .22 mg. of ascorbic acid per gram of fresh vegetable.

Kale was found by Chi and Read (1935) to contain .34 mg. of ascorbic acid per gram; Lunde (1938) found that Norwegian kale contained approximately .75 mg. ascorbic acid per gram. Chi and Read (1935) reported an ascorbic acid value for kohlrabi of .69 mg. per gm. by the indophenol titration; the value given by Ranganathan (1935) was .845.

The vitamin C content of parsley was found to be 1.40 mg. per gm. by Tauber and Kleiner (1935), 1.76 by Bessey and King (1933), and 2.81 by Ranganathan (1935).

TABLE 4

Ascorbic Acid Content of Fresh and Stored Broccoli

(Expressed in mg. per gm.)

Variety	Whole plant (July)				Blossoms (autumn)					
	Freshly harvested		After 2 weeks at 1-3°C. (33.8-37.4°F.)		Freshly harvested		After 2 weeks at 1-3°C.		After 4 days at room temp.	
	Fresh basis	Dry-weight basis	Fresh basis	Dry-weight basis	Fresh basis	Dry-weight basis	Fresh basis	Dry-weight basis	Fresh basis	Dry-weight basis
Italian Early Calabrese	1.27	11.0	1.28	10.5	1.71	9.52	1.46	8.37	.....	.....
Italian Green Sprouting	1.16	11.3	1.29	11.3	1.99	10.1	1.89	9.59	0.98	4.93
Propagane	1.45	13.0	1.41	11.4	1.80	10.4	1.48	8.59	.....	.....
Emerald Isle	1.14	10.5	1.10	10.0	1.85	9.86	1.73	9.35	1.19	6.08
Average	1.26	11.5	1.27	10.8	1.84	9.97	1.64	8.96	1.09	5.51

The purpose of this investigation was to determine the vitamin C content of these vegetables fresh from the field and after storage at different temperatures for varying periods of time, and to study varietal differences. Consideration was also given to determination of the relative ascorbic acid content of the various portions of the plant, and, in the case of cantaloup, the influence of maturity.

TABLE 5

Ascorbic Acid Content of Freshly Harvested Cauliflower

(Expressed in mg. per gm. of fresh vegetable)

Variety	Whole plant			Blossoms		Stems	
	Number of analyses	July	Sept.	Number of analyses	July	Number of analyses	July
White Mountain	9	.94	0.88	2	1.04	2	.82
Forbes	7	.97	0.95	5	1.06	5	.95
Early Snowball	6	.89	1.01	5	1.03	5	.86
Danamerica	9	.95	0.87	4	1.00	4	.90
Average or total	31	.93	0.91	16	1.03	16	.90

EXPERIMENTAL PROCEDURE

The method used to determine the ascorbic acid content of the vegetables was essentially that of Bessey and King (1933) as modified by Mack and Tressler (1937). Many of the analyses were checked in Pittsburgh by the modified method described by Musulin and King (1936). For the cauliflower, broccoli, endive, lettuce, parsley, and New Zealand spinach a mixture of five per cent sulfuric and two per cent metaphosphoric acids was used in the extraction. In the work on cantaloups and parsnips an eight-per cent acetic acid and two-per cent metaphosphoric acid mixture was used. Eight-per cent trichloracetic acid and two-per cent metaphosphoric acid mixture was used for the analysis of kale.

TABLE 6

Ascorbic Acid Content of Fresh and Stored Cauliflower

(Expressed in mg. per gm.)

Variety	Freshly harvested		After 2 weeks at 1-3°C. (33.8-37.4°F.)		After 4 days at room temp.	
	Fresh basis	Dry-weight basis	Fresh basis	Dry-weight basis	Fresh basis	Dry-weight basis
White Mountain	0.94	10.6	0.95	10.9	.54	6.4
Forbes	0.96	9.74	1.00	10.7	.....	.....
Early Snowball	1.03	10.2	1.06	10.9	.....	.....
Danamerica	0.93	10.3	0.98	10.9	.....	.....
Average	0.96	10.2	0.99	10.8	.54	6.4

TABLE 7

Ascorbic Acid Content of Freshly Harvested Endive

(Expressed in mg. per gm.)

Variety	Edible portion		
	Number of analyses	Fresh basis	Dry-weight basis
Green Curled Ruffick	10	.14	2.02
Batavian Full-Heart	13	.12	2.06
White Curled	8	.13	2.04
Green Curled Pancalier	8	.14	1.88
Average or total	39	.13	2.00

TABLE 8

Ascorbic Acid Content of Freshly Harvested and Stored Endive

(Expressed in mg. per gm. of fresh vegetable)

Variety	Freshly harvested		After 9 days at 1-3°C. (33.8-37.4°F.)		After 3 days at room temp.	
	Fresh basis	Dry-weight basis	Fresh basis	Dry-weight basis	Fresh basis	Dry-weight basis
Green Curled Ruffick	.14	1.92	.08	0.512	.04	.367
Batavian Full-Heart	.12	1.61	.10	1.28	.....	.....
Green Curled Pancalier	.14	1.88	.09	1.67	.04	.415
Average	.13	1.80	.09	1.15	.04	.391

TABLE 9

Ascorbic Acid Content of Freshly Harvested and Stored Lettuce

(Expressed in mg. per gm.)

Variety	Number of analyses	Freshly harvested
White Boston	7	.13
Salamander	5	.14
Black Seeded Simpson	4	.21
Mignonette	4	.11
California Iceberg	6	.10
Romaine	6	.19

STORAGE STUDIES

Variety	Freshly harvested			After 10 days at 1-3°C. (33.8-37.4°F.)	
	Number of analyses	Fresh basis	Dry-weight basis	Fresh basis	Dry-weight basis
California Iceberg	2	.12	2.48	.04	1.13
Romaine	2	.15	2.30	.10	1.77
	Freshly harvested			After 3 days at room temp.	
California Iceberg	2	.09	2.00	.06	1.30
Romaine	2	.14	2.28	.08	1.08

In the study of cantaloup, lettuce, kale, and parsnips it was found necessary to treat a second ten-milliliter aliquot of the extract with hydrogen sulfide to ascertain that all the biologically active ascorbic acid was being measured. This procedure is a modification of that proposed by Tillmans, Hirsch, and Jackisch (1932).

For purposes of comparison and to reduce the risk of gross errors in analytical work to a minimum, biological assays were made on samples of most of the vegetables. The samples were frozen and shipped in a container with dry ice to the University of Pittsburgh. All the material was frozen raw, with the exception of the parsnips and cauliflower which were blanched in steam for three minutes and then cooled in cold water before packing in the containers for freezing. Endive for the assay was bought fresh from the market each day because no suitable method could be found for retention of the ascorbic acid after freezing. The curative type of assay, previously described by Tressler, Mack, and King (1936), was used as a measure of the ascorbic acid value.

The moisture content of nearly all the samples was determined, and calculations were made on both a wet and a dry basis. Duplicate samples were taken in all cases. With the cauliflower and broccoli one-fourth of a head was used, the stem being cut off approximately to the edible portion and discarded. One-fourth of a head of the leafy vegetables was used also. Each section was then cut in small pieces and representative samples, obtained by quartering, were taken for the determinations.

Radial sectors of 20 grams were taken from the cantaloups, the edible portion being taken down to the green part. In determining the vitamin C content of the green portion, the netted outside layer was pared off as thinly as possible. The sections were placed in 30 milliliters of the extracting acid mixture and ground immediately with the aid of a small amount of sand.

The endive was sampled by taking one-half of a head, cutting out the core-like base, rolling the leaves up, and cutting them into small pieces; ten-gram portions were then used for each analysis.

Eight to 10 kohlrabi and parsnips were selected for each determination. Quarters were sectioned out lengthwise and cut fine for sampling (10 grams). The skin was left on in all these determinations.

In the investigation of the effects of storage on broccoli, New Zealand spinach, lettuce, cauliflower, and kale, some of the vegetables were kept at a temperature just above freezing (1 to 3°C.) and others were held at room temperature. The procedure for sampling was the same as in the fresh vegetable studies except that the

TABLE 10

Ascorbic Acid Content of Cantaloup

(Expressed in mg. per gm. of fresh vegetable)

Variety	Number of analyses	Edible portion
Bender's Surprise	6	.23
Golden Marvel	4	.38
New Wonderful	4	.24
Cooper's Sweetheart	4	.25
Honey Rock	4	.48
Aristocrat	4	.37

MATURITY STUDIES (Bender's Surprise)

	Number of analyses	Yellow portion	Green portion
Unripe	2	.18	.08
Nearly ripe	2	.31	.14
Ripe	2	.33	.24
Overripe	2	.15	.11

TABLE 11

Ascorbic Acid Content of Freshly Harvested and Stored Parsnips

(Expressed in mg. per gm.)

Month	Pit		1-3°C. (33.8-37.4°F.)		7°C. (44.6°F.)	
	Fresh basis	Dry-weight basis	Fresh basis	Dry-weight basis	Fresh basis	Dry-weight basis
November	.400	.....	.....	.....	.....	.....
December	.216	1.04	.....	.....	.....	.....
January	.202	0.97	.204	.94	.157	.72
February	.165	0.86	.137	.67	.107	.41
March	.149	0.69	.129	.53	.103	.41

TABLE 12

Ascorbic Acid Content of Other Vegetables Studied

(Expressed in mg. per gm.)

Vegetable	Number of analyses	Average value on fresh basis
Parsley	8	1.98
New Zealand spinach	9	0.28-0.59[1]
Kohlrabi	6	0.65
Kale		
Bloomsdale Double Curled	7	1.47
Dwarf German	5	1.12

[1] Value increased with the maturity of plant, apparently owing to enlarging blossoms at base of the leaves.

dry, cut edges were removed to avoid using samples containing portions which had been directly exposed to the air.

The parsnips were stored in the fall of 1937 and were sampled during the winter. Some were placed in a pit and covered with a solid top and a straw mat; others were stored at 1 to 3°C. and at 7°C.(44.6°F.).

The cantaloup study was not begun until late in the season. Varietal differences and changes with increasing maturity in the Bender's Surprise variety were observed.

The results of the analyses are shown in the tables.

DISCUSSION

The results of the biological assays were in good agreement with the titration values. The growth response and scurvy scores for animals receiving the test foods agreed well with those receiving the standard solutions of vitamin C. Where there are apparent differences in the titration values given for the vegetables, the loss in vitamin C occurred while the material for biological assay was in cold storage. Chemical determinations run at the time of the biological assay on the frozen material checked very closely with the results of the assay itself.

Variety was not an important factor in the vitamin C content of broccoli and cauliflower. Loss of ascorbic acid during storage was very slow at temperatures slightly above freezing, over a period of two weeks; but it was very marked in four days at room temperature in summer. Broccoli showed a higher content of vitamin C in the autumn than in the summer. Both broccoli and cauliflower contained more of the vitamin in the blossom than in the stem.

Varietal differences in ascorbic acid content of endive were very slight. This vegetable lost vitamin C rapidly at room temperature but very slowly at refrigeration temperatures.

The fresh-lettuce values were close to those of the endive but the loss was much more rapid during storage, even at low temperatures. The outside leaves contained slightly more of the vitamin than the inner ones.

In the study of cantaloups, distinct differences were noted in the ascorbic acid content of the several varieties. A study of the changes that occurred with increasing maturity in the Bender's Surprise variety indicated that the greatest amount of vitamin C was present at optimum maturity. The green portion of the cantaloups contains less vitamin than the edible portion.

Parsnips decreased in ascorbic acid content as the winter progressed but those in the pit maintained the highest values. Those

at the highest temperature (7°C.) lost their antiscorbutic value most rapidly. The parsnips at the lower temperature remained firm and sweet, but those at the higher temperature became badly wrinkled and were in poor condition.

SUMMARY

Since the data obtained by bioassays of broccoli, cauliflower, endive, and parsnips were in agreement with the results of ascorbic acid determinations by the dichlorophenol indophenol titration technique, the latter procedure is apparently satisfactory for the estimation of differences in ascorbic acid content that may be caused by differences in variety, maturity, freshness, and growing conditions.

TABLE 13

Ascorbic Acid Content of Fresh and Stored Kale and New Zealand Spinach

(Expressed in mg. per gm.)

Vegetable	Freshly harvested		After 2 weeks 1-3°C. (33.8-37.4°F.)		After 4 days at room temp.	
	Fresh basis	Dry-weight basis	Fresh basis	Dry-weight basis	Fresh basis	Dry-weight basis
Kale						
Bloomsdale Double Curled...	1.50	10.34	1.16	7.78	.74	3.55
Dwarf German.....	1.26	8.96	1.07	8.56	.48	1.83
New Zealand spinach.....	0.44	4.85	0.16	1.96	.21	1.71

The approximate average ascorbic acid content of the edible portion of freshly harvested vegetables, calculated in milligrams per gram, was as follows: parsley 2.0, broccoli 1.3, kale 1.3, cauliflower .9, kohlrabi .65, New Zealand spinach .45, cantaloup .35, lettuce .15, and endive .14. The very high ascorbic acid content of parsley, broccoli, kale, cauliflower, kohlrabi, New Zealand spinach, and parsnips is noteworthy.

When held at common room temperatures in summer, broccoli, cauliflower, endive, lettuce, kale, and New Zealand spinach lost ascorbic acid rapidly. In the case of broccoli and cauliflower, refrigeration reduced the loss of vitamin to an almost negligible figure. Refrigeration also aided materially in reducing the rate of loss of ascorbic acid from endive, but it was not so effective in retarding the loss of ascorbic acid from lettuce and kale.

Parsnips lost a large portion of their vitamin C content during winter storage.

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VITAMIN VALUES OF GARDEN-TYPE PEAS PRESERVED BY FROZEN-PACK METHOD. III. THIAMIN (VITAMIN B_1)

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While many investigations on the vitamin C values of frozen fruits and vegetables have been carried on, only a few comparable studies have been reported on thiamin (vitamin B_1) values. Morgan and coworkers (1935) reported that frozen grapes (Thompson seedless) contained only 37 per cent as much vitamin B as the same variety of grapes from the open market. Rose and Phipard (1937) compared the vitamin B values of frozen with fresh peas and found no difference between the raw fresh and frozen peas of one year; however, the frozen peas from the commercial pack of two different years varied greatly. There was no indication as to whether these peas were of the same variety, or whether soil conditions and climate had differed in the two years. Fresh and frozen lima beans varied greatly in vitamin B contents, but the authors attempted no comparison, because the lima beans came from different crops grown in different localities.

Fellers and coworkers (1939) found that there was little loss of vitamin B_1 in the freezing of peas and spinach but that frozen asparagus contained 25 per cent less and frozen lima beans 50 per cent less vitamin B_1 than the fresh vegetable. He attributed these losses to the longer blanching times which are required for asparagus and lima beans.

This investigation was planned to study various factors which might affect the thiamin content of frozen peas. In the first study here reported, the variants were time and temperature of blanching prior to freezing.

The peas were prepared by Mr. H. C. Diehl of the Frozen Pack Laboratory, U. S. Bureau of Chemistry and Soils, Seattle, Washington. They were duplicate lots of those studied by Todhunter and Sparling (1938) and Todhunter (1939) for vitamin A and C values, the results of which studies were recently reported. These peas were harvested, vined, transported to the laboratory as quickly as possible, blanched, cooled, packed into No. 2 cans, sealed without vacuum, and frozen. After being shipped to Corvallis, they were

[1] With the technical assistance of Helen Hayden and Helen Feikert.

stored in the room used for hardening ice cream in a local creamery, where the temperature did not rise above —18°C.(—.4°F.) and usually was much lower. When needed, a container sufficient to last a week was opened and the contents broken up while still frozen, packed into small covered jars, and kept in the freezing unit of an electric refrigerator where the temperature remained about —12 to —15°C.(10.4 to 5°F.).

EXPERIMENTAL PROCEDURE

The peas, all of the Telephone variety, were blanched in hot water at 99°C.(210.2°F.) for one, two, or three minutes; at 88°C. (190.4°F.) for two, four, or six minutes; and at 71°C.(159.8°F.) for two, four, or six minutes. One lot was blanched in steam at 99°C. for one minute; and two lots were held at room temperature

TABLE 1

Growth of Rats on Thiamin-Free Diet Plus Graded Amounts of Thiamin

Level of thiamin fed	Males		Females	
	Number of animals	Gain in weight in 4 weeks	Number of animals	Gain in weight in 4 weeks
micrograms		*gm.*		*gm.*
1	7	—5.86	4	—3.00
2	5	9.60	8	9.25
3	19	21.31	19	23.84
4	10	33.70	9	29.56
5	15	38.73	13	33.70

for four and eight hours, respectively, before being scalded for one minute in water at 99°C.

The thiamin values of the peas were determined by the rat-growth method as described by Chase and Sherman (1931). A curve of response for this colony of rats to graded amounts of crystalline thiamin hydrochloride had been determined by Johnson (1938), and the data gathered by her were recalculated to make logarithmic curves of response for males and females according to the method described by Coward (1938). When the peas were assayed, one litter-mate control was always kept as a negative control with no supplement. Another was given a known amount (three, four, or five micrograms) of thiamin hydrochloride per day, and these gains in weight were included with those obtained by Johnson in constructing the curves of response, as the two investigations were being carried out at the same time and were comparable. The average gains of the animals receiving the different levels of thiamin are given (Table 1).

DISCUSSION OF RESULTS

All animals were raised in this laboratory and were started on the thiamin-free diet at 21 to 28 days of age when weighing 40 to 50 grams. Depletion of body stores of the vitamin was considered accomplished when the animals had reached a state of stationary or declining weight, i.e., when they weighed no more than they had five days previously. They were weighed twice a week for the experimental period of four weeks. The supplements of peas, although calculated on the basis of a daily dose (six days a week), were actually fed three times a week in double portions, as it was found from previous experiment in this laboratory that the two methods of feeding gave the same response. The peas were weighed while still frozen. The total gains in weight for the four weeks were compared with the gains of the animals receiving the thiamin hydrochloride, and the thiamin contents of the peas were calculated from the curves.

The results show that the animals receiving the larger amounts of peas grew to such an extent that it was impossible to convert their gains in weight into thiamin values, as that process would have involved extrapolation of the curve to an unwarranted extent (Table 2). As the gains of animals receiving the larger amounts of peas are fairly consistent with those of animals receiving the smaller doses, the ones receiving these higher levels act as checks on those getting the smaller amounts.

The peas blanched at the lowest temperature, 71°C.(159.8°F.) for two minutes (Lot 1730), had the highest thiamin value, four micrograms per gram. The lowest value was observed with the peas blanched for two and three minutes at 99°C.(210.2°F.), 2.6 micrograms per gram, a significant decrease. In general the trend was towards lower values with increased temperature of blanching, and with longer times; although it is only when the two factors are combined, i.e., higher temperature and longer period of blanching, that the difference is significant. None of the other differences are significant statistically, little difference being noted between steam or hot water blanching at the same temperature, and little effect of holding at room temperature before freezing being noted.

The next study was carried out on peas of different varieties, all grown under identical conditions and frozen after having been blanched at 88 to 93°C.(190.4 to 199.4°F.) for one minute. They were stored and their thiamin values were measured in the same way as the Telephone peas described previously. All these peas were fed at levels of .8 and 1.2 grams per day, with six or more animals in

TABLE 2

Thiamin Contents of Peas Frozen After Different Methods of Blanching

Lot No.	Time of scalding	Temperature of scalding	Number of animals	Level of peas fed	Average gain in 4 weeks	Thiamin content, micrograms per gram, with P.E.	Difference from No. 1730 with P.E.	Critical ratio [1]
	min.	*°C.*		*gm.*	*gm.*			
1723	1	99 [2]	6	0.8	14.1	2.9 ± .34	1.1 ± .50	2.2
1723	....	....	10	1.2	35.8	.....	.....	.....
1724	1	99	6	0.8	18.1	3.3 ± .24	0.7 ± .44	1.6
1724	....	....	9	1.2	43.7	.....	.....	.....
1725	2	99	6	0.8	11.3	2.6 ± .36	1.4 ± .52	2.7
1725	....	....	9	1.2	30.4	.....	.....	.....
1726	3	99	6	1.0	18.2	2.6 ± .18	1.4 ± .41	3.4
1726	....	....	6	1.5	34.8	.....	.....	.....
1727	2	88	6	1.0	24.5	3.4 ± .39	0.6 ± .54	1.1
1727	....	....	6	1.5	48.5	.....	.....	.....
1728	4	88	6	1.0	18.8	2.7 ± .29	1.3 ± .46	2.8
1728	....	....	6	1.5	48.7	.....	.....	.....
1729	6	88	8	0.8	12.3	2.8 ± .30	1.2 ± .47	2.6
1729	....	....	8	1.2	34.8	.....	.....	.....
1730	2	71	8	0.8	23.0	4.0 ± .37	.....	.....
1730	....	....	8	1.2	37.1	.....	.....	.....
1731	4	71	9	0.7	13.9	3.1 ± .18	0.9 ± .41	2.2
1731	....	....	9	1.05	31.7	.....	.....	.....
1732	6	71	9	0.8	16.7	3.1 ± .18	0.9 ± .41	2.2
1732	....	....	9	1.2	36.5	.....	.....	.....
1733	1	99 [3]	9	0.8	15.8	3.0 ± .22	1.0 ± .43	2.3
1733	....	....	10	1.2	32.0	.....	.....	.....
1734	1	99 [4]	9	0.8	14.0	2.9 ± .19	1.1 ± .42	2.6
1734	....	....	8	1.2	32.4	.....	.....	.....

[1] If ratio of difference to the probable error (P.E.) of the difference is three or more, the difference is significant (Chaddock's *Principles and Methods of Statistics*). [2] Blanched in steam; all others blanched in hot water. [3] Held four hours at room temperature before blanching. [4] Held eight hours at room temperature before blanching.

each group. With all varieties but Laxton's Progress, the animals receiving 1.2 grams grew to such an extent that it was impossible to use the curves of response for determining thiamin values. With Laxton's Progress, the growth of those animals receiving .8 gram of peas per day averaged —.5 gram for the four-week experimental period, and therefore the 1.2-gram level of feeding was used as a basis of comparison for this variety.

TABLE 3

Thiamin Contents of Frozen Peas of Different Varieties

Variety	Number of animals	Level of peas fed	Average gain in 4 weeks	Thiamin content, micrograms per gram, with P.E.	Difference from Asgrow 40 with P.E.	Critical ratio [1]
		gm.	*gm.*			
Asgrow 40	9	0.8	40.0	7.4 ± .50	.....	.....
	8	1.2	54.8	.....	.....	.....
World Record	6	0.8	39.8	7.1 ± .75	0.3 ± .90	0.3
	6	1.2	48.3	.....	.....	.....
Alderman	8	0.8	37.8	6.7 ± .30	0.7 ± .58	1.2
	9	1.2	52.4	.....	.....	.....
Rogers 95	7	0.8	37.1	6.5 ± .51	0.9 ± .71	1.3
	7	1.2	49.0	.....	.....	.....
Stratagem	9	0.8	30.5	5.2 ± .38	2.2 ± .63	3.5
	8	1.2	48.9	.....	.....	.....
Extra Early Gradus	9	0.8	29.4	5.0 ± .66	2.4 ± .83	2.9
	8	1.2	45.3	.....	.....	.....
Confidence	6	0.8	28.7	4.3 ± .53	3.1 ± .73	4.2
	7	1.2	46.6	.....	.....	.....
Glacier Park	5	0.8	22.0	3.6 ± .25	3.8 ± .56	6.8
	6	1.2	39.0	.....	.....	.....
Telephone [2]	6	0.8	18.1	3.3 ± .24	4.1 ± .55	7.4
	9	1.2	43.7	.....	.....	.....
Laxton's Progress	8	0.8	—0.5	.....	.....	.....
	8	1.2	16.5	2.0 ± .14	5.4 ± .52	10.4

[1] If the ratio of difference to the probable error (P.E.) of the difference is three or more, the difference is significant (Chaddock's *Principles and Methods of Statistics*).

[2] Lot 1724 above; these peas were not grown in the same place or under the same conditions as the others listed in this table.

The results of this series indicate that these varieties vary widely in thiamin content (Table 3). Asgrow 40, World Record, Alderman, and Rogers 95 all fall into the highest class, with a thiamin content of 6.5 to 7.4 micrograms per gram of peas. The next group, containing 4.3 to 5.2 micrograms per gram, consists of Extra Early Gradus, Stratagem, and Confidence. These are significantly lower values than those for the first group. Glacier Park variety has a still lower value of 3.6 micrograms per gram, and Laxton's Progress

is last with a value of 2.0 micrograms per gram. The Telephone variety listed in the table, containing 3.3 micrograms per gram, is Lot 1724 reported above. This lot was not grown at the same time or in the same place as the other varieties and so is not strictly comparable but is included here as a matter of interest.

SUMMARY

The thiamin values of frozen Telephone peas, all grown under the same conditions of soil and climate, but scalded at different temperatures for different lengths of time prior to freezing, have been determined.

The peas blanched at 71°C.(159.8°F.) for two minutes showed the highest values, having four micrograms thiamin per gram. A general trend was observed towards lower thiamin contents of those peas scalded for the longer times and at the higher temperatures, although this was not a significant difference except for the peas blanched for two and three minutes at 99°C.(210.2°F.), the highest temperature.

No significant difference was found between peas blanched in steam or in hot water at the same temperature for the same length of time, nor for those which were held for four or eight hours at room temperature after vining and before freezing.

Of different varieties grown under the same soil and climatic conditions and all frozen after the same method of processing, Asgrow 40, World Record, Alderman, and Rogers 95 were found to be significantly higher in thiamin values than Stratagem, Extra Early Gradus, and Confidence. In the lowest group were Glacier Park and Laxton's Progress.

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A PROTEOLYTIC ENZYME OF LIMA BEAN (*PHASEOLUS LUNATUS* L.)[1]

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A proteinase of the papain type has been found in a variety of lima beans known as Fordhook. This enzyme occurs in all parts of the immature edible seed but appears to be more abundant in the seed coat than in cotyledon or plumule and hypocotyl tissues. It is present in the dry mature seed and increases on germination (Table 1).

The presence of this proteinase has been demonstrated by its digestion of casein and liquefaction of gelatin. The course of casein digestion was followed at pH 5.0 by the alcoholic titration method of Willstätter and Waldschmidt-Leitz in the manner employed by Balls, Swenson, and Stuart (1935).

EXPERIMENTAL PROCEDURE

A preparation suitable for the purpose of this study was made by extracting the seed coats from freshly gathered, immature, edible lima beans with dilute ammonium sulphate containing a trace of cysteine hydrochloride. Quantities of 100 grams of seed coats to 300 c.c. of cold extractant were finely divided in a Waring liquefier in five minutes to prepare the tissue for extraction in the cold. The protein fraction precipitating between .4 and .8 saturation was purified by washing with acetone, acetone-ether, and drying from ether. This is called "Preparation A."

The results of the casein digestion (Table 2) were quite similar to those recorded by Balls and Hale (1938) for wheat-bran proteinase, in that large titration values were obtained after the long digestion period. This large increase in titration values after 20 hours may be due to an accompanying peptidase, as is thought to be the case with the wheat enzyme.

The gelatin viscosity method of Northrop (1930) was convenient for following the activity of the bean proteinase, either in preparations of the enzyme, such as Preparation A, or in glycerol extracts. Five cubic centimeters of six-per cent gelatin solution buffered at

[1] Food Research Division Contribution No. 435.

pH 5.0 with M/20 citrate was added to five c.c. of water containing the enzyme and an activator or inhibitor in a viscosity tube. Usually 10 milligrams of neutralized cysteine-hydrochloride were allowed to act 30 minutes for activation. The temperature was 35.5°C.(96°F.).

The glycerol extracts were made by grinding the tissue, with sand and 50 per cent glycerol, in a mortar. Where possible, a proportion of one gram of tissue to two c.c. of extractant was maintained.

TABLE 1

Proteinase of Tissues of Seed of Lima Bean

(Mg. of reference enzyme per c.c. of glycerine extract representing 500 mg. of tissue.)

Immature edible seed		
Plumule and hypocotyl	Seed coat	Cotyledon
30	60	36

Mature whole bean	
Dry	25
Sprouted	78

The results by the viscosity method are expressed in milligrams of a crude dry enzyme preparation made from the seed coats of the immature edible beans. A set of empirical curves (Fig. 1) was made by measuring the loss in viscosity induced in gelatin by various quantities of the crude preparation at intervals of 15, 30, 45, and

TABLE 2

Casein Digestion by Bean Proteinase at 40°C.(104°F.)

Amount of enzyme	Set-up	N/10 KOH after	
		2 hours	20 hours
c.c.[1]		*c.c.*	*c.c.*
None	Casein alone	0.00	0.00
2	No casein	0.00	0.00
2	Casein + 10 mg. cysteine	0.90	3.62
4	Casein + 10 mg. cysteine	1.40	5.70

[1] Each c.c. contained 25 mg. of Preparation A.

60 minutes. From the loss in viscosity, the quantity of enzyme in an unknown preparation or extract can be estimated.

The crude preparation adopted as a basis for the reference curves was made by subdividing the seed coats in cold acetone, containing a trace of cysteine, in the Waring liquefier. After washing with acetone-ether, the tissue was dried from ether.

The enzyme is not wholly active either in Preparation A or in glycerine extracts of the whole bean (Table 3). Cysteine, KCN,

and H_2S activate the enzyme. Iodoacetic acid, bromate, thiosulphate, and hydrogen peroxide act as inhibitors.

The preceding description indicates that the nature of the enzyme is similar to that of papain. The principal purpose of this paper, however, is to describe the action of the enzyme in the bean itself, particularly with reference to the behavior of quick-frozen beans stored at low temperature.

Experiments were accordingly planned to test the effect of freezing, blanching, and behavior on the autolytic breakdown of the

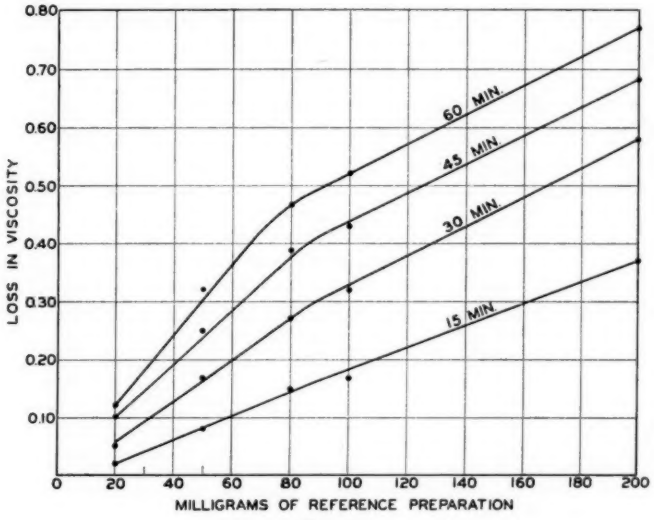

FIG. 1

protein. For the autolysis freshly picked beans were finely divided in the Waring liquefier. A proportion of three c.c. of water to one gram of bean tissue was used. This mixture preserved with toluol was kept at 40°C.(104°F.) in a bottle which was open to the air only through a capillary tube. At intervals, aliquots of the well-stirred mixture were removed for pH and protein nitrogen determinations, and for formol titrations. The nitrogen of the centrifuged autolysate was determined by the micro-Kjeldahl method after precipitation in 2½ per cent trichloracetic acid.

The data from autolysis (Table 4) show how much blanching reduces the rate and extent of the activity of the proteinase in the whole bean. Not only the rate of autolysis but also the quantity of protein going into solution are sharply reduced by the three-minute

TABLE 3

Activation and Inhibition of Lima Bean Proteinase

(Mg. of reference enzyme)

Acetone preparation A			
Not activated	Activated with 4 mg. cysteine	Activated with 4 mg. cysteine, then treated with M/100 iodoacetic acid 10 min.	2M. KCN Activation
166	208	113	174

Glycerol extract of whole bean						
Not activated	Activated	H_2S activation	Activated, then treated with M/100 iodoacetic acid 10 min.	Activated by 10 mg. cysteine, then treated 10 minutes with		
				2 mg. thiosulphate	1 mg. potassium bromide	1 c.c. N/10 hydrogen peroxide
24	42	44	31	23	29	33

TABLE 4

Autolysis of Lima Bean[1] *at 40°C.(104°F.)*

Time from start of autolysis	Untreated			Frozen-thawed			5 sec. blanch		
	N	Base	pH	N	Base	pH	N	Base	pH
Nov. 15	*mg.*	*c.c.*		*mg.*	*c.c.*		*mg.*	*c.c.*	
4 hours	1.93	0.64	6.30	1.97	0.61	6.35	2.04	0.54	6.30
68 hours	1.26	0.85	6.12	1.51	0.77	6.13	1.37	0.71	6.10
7 days	1.32	0.97	6.15	1.48	0.90	6.13	1.31	0.80	6.09
13 days	1.30	1.04	6.13	1.19	1.14	5.32	1.28	0.85	6.05
20 days	1.31	1.08	6.01	0.96	1.32	4.90	1.18	0.98	5.40

Time from start of autolysis	45 sec. blanch			3 min. blanch			5 min. blanch		
	N	Base	pH	N	Base	pH	N	Base	pH
Nov. 15	*mg.*	*c.c.*		*mg.*	*c.c.*		*mg.*	*c.c.*	
4 hours	2.24	0.43	6.50	0.79	0.25	6.58	0.42	0.21	6.58
68 hours	1.84	0.43	6.30	0.90	0.26	6.48	0.54	0.21	6.50
7 days	1.90	0.48	6.28	0.95	0.28	6.50	0.55	0.21	6.50
13 days	1.69	0.49	6.21	0.84	0.30	6.41	0.54	0.24	6.48
20 days	1.44	0.48	6.10	0.83	0.29	6.30	0.53	0.25	6.32

Time from start of autolysis	Seed coat			Cotyledon			Plumule and hypocotyl		
	N	Base	pH	N	Base	pH	N	Base	pH
	mg.	*c.c.*		*mg.*	*c.c.*		*mg.*	*c.c.*	
4 hours	0.05	0.80	5.84	3.22	0.65	6.50	1.06	0.93	6.75
5 days	0.03	0.96	5.50	3.00	0.72	6.51	0.92	1.16	7.10
13 days	.....	1.02	5.55	2.89	0.77	6.53	0.80	1.29	7.35
21 days	.....	0.96	5.58	2.94	0.81	6.44	0.85	1.41	7.28

[1] Except for seed coat and plumule-hypocotyl, each sample contained 100 grams of immature, edible bean. The frozen-thawed sample was held for two days at —17.8°C.(0°F.) before thawing. Milligrams of N and c.c. of N/20 base are reported per c.c. of centrifuged autolysate and pH by glass electrode.

and five-minute periods of blanching. Although the heat treatment in itself would be expected to make the protein more digestible by denaturing it, there is little evidence to indicate increased enzyme activity because of a change in the nature of the protein. The slightly increased activity after 45 seconds of blanching may be due to such a denaturing action. The enzyme itself appears to be sensitive to heat.

No evidence of marked acceleration of enzyme activity is present in the frozen sample. The effects of freezing and blanching, as shown by autolysis (Table 4), may be compared with the effects of freezing and blanching as determined by the viscosity method (Tables 5 and 6). In view of the variability of different samples from the same lot, and perhaps because of the influence of short storage at 3.3°C.(38°F.), conclusions should be drawn only after the accumulation of more data, particularly on the effect of freezing.

TABLE 5

Effect of Blanching on Proteinase of Lima Beans

(Mg. of reference enzyme preparation per c.c. of glycerol extract found after 30 minutes' activation, by viscosity method)

Oct. 24	Not blanched	76
Oct. 24	Blanched 15 seconds in boiling water	17
Oct. 24	Blanched 45 seconds in boiling water	15
Oct. 24	Blanched 3 minutes in boiling water	10
Oct. 27	Stored at 3.3°C.(38°F.) for two days	60
Nov. 15	Not blanched	69
Nov. 15	Blanched 10 seconds	23
Nov. 15	Blanched 15 seconds	18
Nov. 15	Blanched 45 seconds	10

Preliminary work has been started to investigate the possible occurrence of an hydrocyanic acid-containing glucoside, with an accompanying enzyme, reported to be present in lima beans by Henry and Auld (1908).

The relationship of naturally occurring hydrocyanic acid to proteinase and to flavor of quick-frozen beans is of interest. This work will be described in another paper.

SUMMARY

A proteinase of the papain type has been found in lima beans. It appears to be similar in nature to wheat proteinase. The bean proteinase appears to be so sensitive to heat that deterioration in well-

blanched, quick-frozen, cold-stored beans is unlikely to be due to proteinase.

CONCLUSIONS

While it is uncertain how far conclusions drawn from autolysis of mixed tissues can be applied to the behavior of proteinase in

TABLE 6

Effect of Freezing on Proteinase of Lima Beans

(Mg. of reference enzyme preparation per c.c. of glycerol extract found after 30 minutes' activation, by viscosity method)

Oct. 3	Not frozen	62
	Not frozen	56
	Not frozen	60
	Not frozen	76
	Not frozen	76
	Average	66
Oct. 4	Held frozen at —17.8°C.(0°F.) over night	60
	Held frozen at —17.8°C.(0°F.) over night	60
	Held frozen at —17.8°C.(0°F.) over night	50
	Average	57
Oct. 4	Not frozen—stored at 3.3°C.(38°F.) one day	58
	Frozen	46
	Frozen	40
Oct. 5	Not frozen, stored at 38°F. for two days	39
	Not frozen, stored at 38°F. for two days	45
	Not frozen, stored at 38°F. for two days	46
Oct. 6	Not frozen, stored at 38°F. three days	56
	Not frozen, stored at 38°F. three days	72
	Average	64

cold-stored intact beans, these results do not appear to indicate that deterioration of well-blanched lima beans can be due to proteinase activity. This conclusion has been reached because the proteinase is reduced by a relatively short blanching period, and because there is little evidence of protein denaturation by heat, which would allow quicker enzymatic breakdown of the protein.

ACKNOWLEDGMENT

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GASEOUS PRODUCTS OF CUCUMBER PICKLE FERMENTATIONS[1,2]

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The normal fermentation of cucumber pickles in salt brines is accompanied by the evolution of considerable quantities of gases. At times during the production of gases, a froth may be formed on the surface of the brine to a depth of as much as four or five inches. The presence of carbon dioxide in these gases has been mentioned in the literature, but no systematic study of them has been reported. A study of the gases evolved was undertaken at a temporary field laboratory located at a commercial cucumber pickling plant at Faison, North Carolina. At this plant facilities were provided for the experimental salting of cucumbers using brines of various salt concentrations; 85-bushel vats were used for the purpose.

The evolution of gases from vats has been taken more or less for granted by commercial cucumber salters. It has been used as an index to the rate of fermentation, and when exceptionally large amounts of gas were evolved, the salt concentration of the brine has been increased to decrease the rate of fermentation. When these studies were first undertaken, analyses for carbon dioxide and oxygen were made and it was found that in some cases during active fermentation the carbon dioxide content of the gases was above 99 per cent. It soon became apparent, however, that at times some other gas besides carbon dioxide was being formed because some samples collected during rapid evolution of gases contained less than 50 per cent carbon dioxide and no oxygen. It was then found that the bubbles on the surface of some brines would explode with a distinct pop when touched with a lighted match. Changes were made in the gas-analysis outfit so that combustible gases could be determined. The combustible gas was found to be hydrogen, but it was also found that hydrogen was not produced by all the cucumber-pickle fermentations. The effect of various factors on the production

[1] Presented at the 97th meeting of the American Chemical Society, Baltimore, Maryland, April 3-7, 1939.

[2] This work was done as a part of a coöperative project between the Bureau of Chemistry and Soils, and the Department of Horticulture, North Carolina Agricultural Experiment Station.

[3] Food Research Division contribution No. 423.

of carbon dioxide and hydrogen, and a study of the microörganisms responsible for the production of these gases proved interesting.

ANALYTICAL METHODS

The gases were analyzed in an apparatus similar to the Williams gas analysis outfit, Model B. Carbon dioxide was determined by absorption in a concentrated solution of potassium hydroxide and noting of the decrease in volume. Oxygen was determined by absorption in alkaline pyrogallol solution. Hydrogen was determined by burning with added oxygen in an electric spark explosion burette and noting the decrease in volume. The absence of methane or other combustible hydrocarbon gases was shown by passing the products of combustion through the potassium hydroxide to absorb any carbon dioxide and noting no decrease in volume. Adjustments in the oxygen-sample ratio nearly always resulted in an explosion. Amounts of hydrogen as low as .3 and .2 per cent were determined by this method.

PRELIMINARY EXPERIMENTS

Cucumbers have small bubbles of gas included in their structure which give a rather opaque, white appearance to the sliced fruit. As the cucumbers cure in brine these bubbles disappear and leave the tissues somewhat translucent. These bubbles may be removed by the repeated application of vacuum or by heating. In order to obtain an approximation of the amount and composition of this gas, a flask was filled with cut cucumbers, water was added, and the water boiled slowly. The gases driven off were collected over saturated brine, measured, and analyzed. A kilogram of cucumbers treated in this manner yielded 94 cubic centimeters of gas of which 37.6 cubic centimeters were carbon dioxide and 1.4 cubic centimeters were oxygen. An analysis for hydrogen was not made, but the remainder was undoubtedly nitrogen. This method may be subject to some criticism, but at least it gives an idea of the extent and composition of the gas in fresh cucumbers. These gases, from the interiors of the cucumbers seem to come out at a fairly constant rate during the fermentation; and nitrogen from this source probably accounts for the sum of the carbon dioxide, oxygen, and hydrogen falling short of 100 per cent, especially during slow evolution of gases.

Although no attempt was made to measure the quantity of gas evolved during the fermentations in vats, a laboratory experiment gives some idea as to the amount of gas evolved. A three-liter flask was packed with cucumbers and allowed to ferment in a brine of 15 per cent saturation for four days and then salt was added to bring the salt content to 30 per cent saturation. During the fermentation

4,800 cubic centimeters of gas were collected, of which 4,600 cubic centimeters were carbon dioxide. No analyses for hydrogen were made. In this case the volume of the gas was slightly over one and one-half times the volume of the fermenting brine plus cucumbers. This treatment corresponds roughly to the 20° salometer treatment explained later. It was observed that vat fermentations in 40 and 60 per cent saturated brines gave off considerably larger volumes of gas than fermentations in brines of 20 and 30 per cent saturation; and since there were variations with the same treatment, this can be considered only a very rough estimate.

EXPERIMENTAL PROCEDURE

In general, commercial practices were followed in salting the cucumbers in the experimental vats. The vats were filled with cucumbers, a layer of boards was fastened in place over the cucumbers, and salt brine added to a level about four inches above the layer of boards. Brines of 20, 30, 40, 60, and 80° salometer were used to cover the cucumbers. The degrees salometer of a brine indicates the percentage of saturation of the brine with respect to salt. Thus, a 100° salometer brine would be saturated and contain 26.4 per cent sodium chloride. Additions of salt were made to the vats to maintain the brines at the indicated salometers during the first week, and then salt was added to the 20, 30, and 40° salometer brines so as to raise the concentrations to 70° salometer by the end of the sixth week. The 60° brine was raised to 70° salometer during the sixth week. The 80° salometer brine was maintained at that concentration. The salting procedures will be indicated by the initial brine concentrations. The temperatures of the brines during the fermentations were within a few degrees of 26.7°C.(80°F.). Most of the vats were outside and unsheltered. Three sheltered vats will be indicated. No special starter or inoculation was used. The cucumbers were not washed and undoubtedly an abundance of microörganisms of various types were introduced by the cucumbers and adhering particles of soil. Inverted funnels were placed over cracks in the layer of boards that held the cucumbers immersed in the brine, and samples of the evolving gases collected in the funnels by displacement. The samples were transferred to the gas-analysis outfit for analysis.

A determination for oxygen was made on all samples, but the values are omitted from the tables. The values were too small to be of significance. During the rapid evolution of gases little or no oxygen was detected. The amounts ranged from none to two or three tenths of one per cent. It is believed that the oxygen came largely from the small bubbles in the interiors of the cucumbers.

Larger amounts of oxygen were an indication that something was wrong with the sample, and the oxygen determination served as a check to indicate whether any air had been introduced into the samples.

RESULTS OF GAS ANALYSES

Some of the results of the gas analyses with the 60° salometer brine fermentations are given (Table 1). The gas collected on the second day probably contained a large amount that had been trapped in the vat during filling and also some gas coming from the interiors of the cucumbers, as indicated previously. From the second to the

TABLE 1

Analyses of Gases Produced by Fermentation of Cucumbers in 60° Salometer Brines

Age	Vat 7		Vat 11	
	CO_2	H_2	CO_2	H_2
days	*pct.*	*pct.*	*pct.*	*pct.*
2	45.0	0.0	38.7	0.0
12	33.9	56.2	17.8	35.4
15	60.4	39.1	41.4	35.8
19	52.3	40.4	51.5	40.8
23	65.0	9.1	72.8	20.5
27	87.4	10.0	79.8	15.1
31	88.7	8.8	93.7	3.9
37	90.2	8.5	89.2	7.1
41	78.8	4.1	81.4	12.2
45	68.7	24.4	82.7	9.3
50	70.5	18.0	90.7	4.4

12th day the evolution of gas was so slow that there was an insufficient amount for analysis. On about the 12th day the evolution of gas started and continued until after the 50th day, when the analyses were discontinued. The most important result to be noted is the persistence of a relatively high percentage of hydrogen throughout the fermentations. During the early part of the active fermentation the content of hydrogen ranged around 40 per cent. On about the 21st day the percentage of hydrogen began to decrease.

Some of the analyses of gases from fermentations in 40° salometer brines, with the different treatments indicated (Table 2), show that hydrogen was found in only one vat, No. 14, and it was found as long as the evolution of gases continued from that vat. Even in the case of this vat the percentage of hydrogen was much less than in the cases with 60° salometer brines. The brines in Vats 6 and 10 were circulated for an hour a day with a pump and it was noted that gases were given off over a shorter period of time from these

TABLE 2

Analyses of Gases Produced by Fermentation of Cucumbers in 40° Salometer Brines

Age	Vat 1 Regular		Vat 14 Regular		Vat 2 Sheltered		Vat 5 Sheltered, stirred		Vat 6 Sheltered, circulated		Vat 10 Circulated	
	CO_2	H_2	CO_2	H_2	CO_2	H_2	CO_2	H_2	CO_2	H_2	CO_2	H_2
days	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*
2	56.3	0	46.9	0.0	49.1	0	43.0	0	39.2	0	44.2	0
7	66.8	0	54.1	21.6	70.7	0	54.8	0	51.1	0	56.4	0
10	53.0	0	60.7	14.0	35.6	0	59.7	0	57.5	0	38.8	0
14	91.0	0	91.0	0.3	61.3	0	43.5	0	52.0	0	91.6	0
20	85.4	0	82.4	1.7	80.7	0	89.9	0	89.0	0	88.2 [1]	0 [1]
24	87.0	0	92.2	1.1	76.2	0	90.7	0	.....	....	.....	....

[1] Age 17 days.

vats than from uncirculated vats with the same brine concentration. Gas evolution continued until about the 30th day from the four uncirculated vats as compared with more than 50 days from 60° salometer brines.

Analyses of gases from fermentations in 20 and 30° brines indicate that both fermentations in 30° brines produced hydrogen and only one in the 20° brines (Table 3). The amount of hydrogen produced in one 30° fermentation, vat No. 9, was very small. In no case was the production of hydrogen as great as that in 60° salometer brines. The production of gases from these vats was less and extended over a shorter period of time than that from vats containing

TABLE 3

Analyses of Gases Produced by Fermentation of Cucumbers in 20 and 30° Salometer Brines

Age	20° Salometer brine				30° Salometer brine			
	Vat 12		Vat 8		Vat 9		Vat 13	
	CO_2	H_2	CO_2	H_2	CO_2	H_2	CO_2	H_2
days	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*
2	63.1	0.0	63.6	0	63.1	0.0	47.4	5.1
5	75.0	0.0	69.5	0	71.4	0.0	51.8	20.0
8	43.2	1.4	52.8	0	61.8	0.0	35.3	7.6
11	71.6	0.7	70.2	0	.....	.....	.....	.....
15	69.2	0.6	55.1	0	76.5	0.3	60.3	9.4
17	59.4	0.5	.....	....	84.3	0.1	72.2	5.7
19	.....	.....	53.4	0	89.4	0.0	75.8	1.9
23	43.9	0.0	.....	....	.....	.....	66.7 [1]	2.1 [1]
27	.....	.....	.....	....	81.6	0.0	60.7 [2]	1.3 [2]

[1] Age 22 days. [2] Age 26 days.

40 and 60° salometer brines. It was noted that the sum of the percentages of carbon dioxide and hydrogen, or the percentage of carbon dioxide alone in cases where no hydrogen was produced, lacked considerable of making 100 per cent. This was probably due to nitrogen from the interiors of the cucumbers making up a large percentage of the gas samples during the slow evolution of fermentation gases.

Several analyses from additional vats (Table 4) serve to emphasize the preceding data. Single analyses from 11 additional 40° salometer vat fermentations are shown. Of these only three showed the presence of hydrogen. R8V6, a regular commercial vat, was interesting. It was gassing at an exceptionally rapid rate and attention was called to it by the plant manager. When a lighted match was applied to the surface bubbles, the bubbles exploded vigorously.

The two analyses of the gases from fermentations in 60° salometer brines show considerable quantities of hydrogen in keeping with the

results shown above. The analysis of the gas from an 80° salometer fermentation is included. The evolution of gas was very slow from this vat; the sample of gas was small and collected over several days' time. Nevertheless, it shows the presence of a considerable proportion of hydrogen.

Analyses were also made of the gas from hollow cucumber pickles. These hollow cucumbers are commonly called "bloaters" by the trade and are formed during the fermentation. Gases appear in the cucumbers and the three carpels separate in the interior. The separation does not extend to the outside of the cucumbers. The gas-filled cavity may extend until it comprises as much as three-fourths of the volume of the cucumber, and at times the fruits are under tension owing to gas pressure from within. This condition occurs more frequently with the large-sized cucumbers. When the cucumbers are allowed to remain in the brine for some time, brine gradually replaces the gas.

TABLE 4

Analyses of Gases From Fermentation of Cucumbers in Brines of Various Concentrations

Vat No.	Age	Brine concentration	Gas composition	
			CO_2	H_2
	days	*° sal.*	*pct.*	*pct.*
193	12	40	95.0	0.0
178	12	40	58.5	0.0
191	12	40	83.5	0.0
187	12	40	79.4	6.5
4-37	16	40	93.1	0.0
6-37	22	40	90.9	0.0
7-37	12	40	90.5	0.0
9-37	23	40	86.2	0.0
10-37	15	40	97.5	0.0
R8V6	2	40	59.0	37.2
R16V6	10	40	99.4	0.2
1-37	21	60	63.8	30.3
5-37	16	60	31.6	55.5
8-37	28	80	21.4	33.3

The analyses of gases from "bloaters" compared with analyses of gases collected at the top of the vats at the same time show a marked similarity in compositions (Table 5). When hydrogen was found in surface gas, it was also found in the "bloater" gas in approximately the same proportion. The percentages of carbon dioxide were also found to agree quite well. Undoubtedly these gases were formed within the cucumbers. However, since "bloaters" were

found in vats from which there was no evolution of hydrogen as well as in vats from which hydrogen was evolved, a fermentation-forming hydrogen within the cucumbers cannot be considered the sole cause of "bloater" formations.

It was also shown that gases were produced in the body of the brine itself. Some brine from an active fermentation-producing hydrogen and carbon dioxide was drawn off into a flask and allowed to ferment. The gas produced in this flask was similar in composition to that collected in the usual manner. It seems that the gases are produced in both the interiors of the cucumbers and in the brine, and that the gases from both sources are similar in composition.

TABLE 5

Analyses of Gases From Hollow Cucumbers Compared With Analyses of Gases Collected in the Usual Manner

Vat No.	Age	Brine concentration	Gas from hollow cucumbers		Corresponding surface gas	
			CO_2	H_2	CO_2	H_2
	days	*° sal.*	*pct.*	*pct.*	*pct.*	*pct.*
3-37	17	20	31.4	0.0	25.9	0.0
13-38	18	30	83.2	0.6	75.8	1.9
4-37	17	40	91.4	0.0	93.1	0.0
6-37	22	40	92.0	0.0	90.9	0.0
1-38	18	40	90.6	0.0	89.2	0.0
14-38	18	40	91.4	0.7	89.0	1.6
1-37	24	60	35.6	29.9	60.8	19.2
1-37	24	60	61.8	31.4	60.8	19.2
5-37	16	60	48.7	44.7	31.6	55.5
7-38	16	60	46.0	42.7	52.3	40.4

BACTERIOLOGICAL STUDIES

Bacteriological studies during the past three years have shown a definite correlation between the carbon dioxide formation during the latter part of the active fermentation and the presence of a typical yeast fermentation. This was especially true of the fermentations in 40° salometer brines, in which typical yeast fermentations started on about the 10th day and continued for about one week. During this period yeast counts of 10,000 to 100,000 per cubic centimeter were obtained.

Bacteriological studies were undertaken to ascertain if microörganisms were responsible for the production of hydrogen. Vat R8V6, mentioned previously as producing large amounts of hydrogen, was thought to present an ideal opportunity for this study. This vat was gassing rapidly during the initial stage of the fermentation. Bacteriological plate counts were made and these indicated the following

numbers of organisms per cubic centimeter: 22 million acid-formers, 200 thousand peptonizers (soil group), and 200 yeasts. Numerous isolations of typical colonies of acid-formers were made and transferred into fermentation tubes using cucumber-juice broth as the liquid medium. None of the resulting fermentations showed the production of gas. It was assumed that the predominating organisms, those occurring in millions per cubic centimeter, were not responsible for the hydrogen production. The ordinary routine plating methods as used for following the brine fermentations were not suitable for the isolation of hydrogen-producing organisms.

Later on other vats were used in a more intensive effort to cultivate the microörganisms responsible for the hydrogen production. This involved numerous platings and the employment of various types of solid media both aerobically and anaerobically. Finally it was demonstrated that the causitive agent could be isolated from the brine by culturing under strict anaerobic conditions. Plate counts indicated that these organisms were present in low numbers as compared with the total count. Cultures isolated and inoculated into Smith fermentation tubes using cucumber-juice broth showed rapid gas production in 48 hours. This gas was analyzed and found to contain a relatively high percentage of hydrogen. These organisms are being studied further in regard to their cultural characteristics and identification.

It should be noted that this work was done under southern conditions. The fermentations in vats took place largely during the month of July at temperatures close to 26.7°C.(80°F.). In the northern part of the United States, such as in the Great Lakes region, the season is much later. The crop there is harvested in August and September and the fermentations occur largely during the relatively cool months of September and October. Somewhat different methods of brining are used in these regions. Usually the strength of the brine is not increased as rapidly as in southern sections. Changes in conditions like these might have considerable effect on the gas production by the fermentation.

SUMMARY

Carbon dioxide was formed during the fermentation of cucumber pickles in brines ranging from 20 to 80 per cent saturation with respect to salt.

Larger quantities of gases were evolved during fermentations in 40 and 60 per cent saturated brines than in brines of 20, 30, and 80 per cent saturation.

Hydrogen was produced in considerable amounts by all the fermentations observed in 60 per cent saturated brines. Hydrogen was produced in smaller amounts by some fermentations in 20, 30, and 40 per cent saturated brines; however, hydrogen was not produced by all fermentations at these brine concentrations.

Organisms capable of producing hydrogen have been isolated from brines.

Gases formed in the interiors of hollow cucumbers or "bloaters" had approximately the same composition as gases collected at the tops of vats from which they were taken.

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